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“Connecting worlds – a view on microfluidics for a wider application”

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Abstract

From its birth, microfluidics has been referenced as a revolutionary technology and the solution to long standing technological and sociological issues, such as detection of dilute compounds and personalized healthcare. Microfluidics has for example been envisioned as: (1) being capable of miniaturizing industrial production plants, thereby increasing their automation and operational safety at low cost; (2) being able to identify rare diseases by running bioanalytics directly on the patient's skin; (3) allowing health diagnostics in point-of-care sites through cheap lab-on-a-chip devices. However, the current state of microfluidics, although technologically advanced, has so far failed to reach the originally promised widespread use.

In this paper, some of the aspects are identified and discussed that have prevented microfluidics from reaching its full potential, especially in the chemical engineering and biotechnology fields, focusing mainly on the specialization on a single target of most microfluidic devices and offering a perspective on the alternate, multi-use, "plug and play" approach. Increasing the flexibility of microfluidic platforms, by increasing their compatibility with different substrates, reactions and operation conditions, and other microfluidic systems is indeed of surmount importance and current academic and industrial approaches to modular microfluidics are presented. Furthermore, two views on the commercialization of plug-and-play microfluidics systems, leading towards improved acceptance and more widespread use, are introduced. A brief review of the main materials and fabrication strategies used in these fields, is also presented. Finally, a step-wise guide towards the development of microfluidic systems is introduced with special focus on the integration of sensors in microfluidics. The proposed guidelines are then applied for the development of two different example platforms, and to three examples taken from literature.

With this work, we aim to provide an interesting perspective on the field of microfluidics when applied to chemical engineering and biotechnology studies, as well as to contribute with potential solutions to some of its current challenges.

Keywords: microfluidics, biotechnology, plug-and-play, sensor integration, platform development guide, modular microfluidics

Glossary

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1. Introduction

Nowadays, the use of micro- or nanofluidics is quite wide-spread across several academic fields, from proteomics and drug discovery to waste management and point-of-care (POC) diagnostics (Becker, 2009), (Mark et al., 2010), (Chiu et al., 2017). Since the first proof-of-concept (Terry et al., 1979), the rapidly increasing interest in the micro- or nanofluidics technology can be associated with its advantages, such as the low manufacturing cost, reduced usage of reagents during operation and the possibility for parallelization and automation (McDonald et al., 2000), (Erickson and Li, 2004). These characteristics along with a highly automated control of the fluidic flow, an easy tuning of temperature and concentration diffusion gradients, as well as the available semiconductor technology, have pushed the interest towards miniaturization of unit operations, together with development of suitable analytical devices and techniques. This subsequently led to an effort towards the integration of these miniaturized units in a single device.

Microfluidics has contributed to the advancement of science by, for example, allowing the fabrication of (new) functional materials (e.g. nanofibers, liposomes) (Daniele et al., 2015), (Han et al., 2017), the isolation and use of unstable or short-life compounds (Yoshida, 2010), (Hessel et al., 2013), single cell monitoring and analysis (Zare and Kim, 2010), (Grünberger et al., 2014), membrane free fuel cells (Wang et al., 2014), all of which are based on phenomena achievable only with microfluidics. More specifically, the accurate control of fluid(s) shape and velocity achievable with the existing diversity of microchannel geometries (e.g. concentric flow, 3D hydrodynamic focusing) has enabled the emergence of microfluidic fiber fabrication towards novel fiber constructs that better mimic human tissue (Daniele et al., 2015). In flow chemistry, the significant reduction of reaction residence time (to the s or ms order of magnitude) due to the attainable decreased dimensions allow the use of highly instable compounds in organic reactions that are performed more safely in this way (e.g. Swern-Moffat oxidation at ambient temperature (Yoshida, 2010)). In both these applications, the ability to control diffusion has had a significant influence, but in the case of microfuel cells it removes the need for integrated membranes thus increasing the fuel/media flexibility (Wang et al., 2014).

However, few of these unique applications of microfluidics have been applied to or been used in commercial systems. The characteristics that were once so appealing in microfluidics, are now more critically analyzed and the application of microfluidics depends on whether those characteristics can provide better performance, usability or information than conventional technologies (Chiu et al., 2017).

1.1 Microfluidics and the chemical engineering and biotechnological market

Initially, the research and development activities in the microfluidics field focused on areas where the highest potential for short-term commercial success was expected (Erickson and Li, 2004). For example, pharmaceutical companies started performing initial screening tests in lab-on-a-chip devices since these presented less false positives and thus higher quality results (Haber, 2006). Despite the obvious potential, microfluidics has still a very small impact in the biotechnological/analytical market today (Mark et al., 2010), (Chiu et al., 2017). Current fields of commercial application of microfluidics mostly involve *in vitro* diagnostics (e.g.

DNA/RNA hybridization and PCR) (Chin et al., 2012), (Ishii et al., 2013), (Volpatti and Yetisen, 2014), pharmaceutical applications (such as drug discovery and screening, as well as drug delivery) (KANG et al., 2008), (Neuži et al., 2012), bio-based production processes (e.g. on and in-line process monitoring of fermentations and biocatalytic reactions) (Fernandes, 2010), (Hegab et al., 2013), (Žnidaršič-Plazl, 2017), and ecology (e.g. water quality assessment and biological threat detection) (Mairhofer et al., 2009), (Bridle et al., 2014). Even though a few microfluidic-based technologies or solutions that include microfluidic parts are available in the market (Bieringer et al., 2013), (Sharma et al., 2015), they are mainly used in research laboratories (Chiu et al., 2017). It is therefore important to reflect on the reason for this relatively low impact, which stands in sharp contrast with the original sky-high ambitions for this field.

When investigating the scientific literature, it is mentioned that this relatively low impact is due to the systems complexity, frequently observed repeatability issues of the existing platforms and the low application flexibility of the majority of the developed microfluidic systems. As discussed by Sackmann *et al.* (2014) for the chemotaxis field, the low adoption of microfluidics by non-specialists (e.g. biologists) has been related with the lack of fluid handling expertise and infrastructures required for their fabrication and/or operation (Sackmann et al., 2014). Also, in some fields, current microfluidic devices may not offer sufficient new or improved capabilities to motivate a change from the field's standards to microfluidic-based assays (Sackmann et al., 2014). For example, the current lifetime of a microfluidic device or microfluidic-based platform, due to debris accumulation, trapped air and biofouling, is too short, compared with standard benchtop equipment, to be economically or technically attractive to non-microfluidic experts (Shields et al., 2017). In a field such as analytics, the main driver towards the use of microfluidic or microstructured systems, will be the possibility of achieving a significantly lower limit of detection. This is especially relevant when the need for such a low limit (e.g. early disease detection, environmental detection of hazardous compounds or microorganisms) is more pertinent than potential reproducibility issues or difficulty in operation. Another barrier to microfluidic systems commercialization may be the required level of investment in order to change the current infrastructure of the target industries (Shields et al., 2017), meaning the adoption of new standards and substitution of equipment. Shields *et al.* (2017) present a good overview on the barriers to commercialization and clinical translation, such as the need for use of intellectual property licensing agreements which limits operability and discourages investment, found in the cell separation microfluidic field (Shields et al., 2017). The manufacturing costs and the required initial investment, which translate into high costs of the final devices, lead to a specific and closed company-customer based business model, instead of a general model relying on publicly available and well described systems that can be combined in order to address a wide range of specific customer needs (Mark et al., 2010). As found by Panikowska *et al.* (2016) in an extensive survey to microfluidic developers both in academia and in industry, there are five basic models for microfluidic system design and development, where prototype development is crucial, but fluidic simulations may be used for design optimization and testing. The microfluidic design is technology based, mostly guided by the available fabrication technologies, but also by the specific application required by the client (Panikowska et al., 2016). Panikowska *et al.* (2016) also found that the view on design flexibility and

customization varies greatly between microfluidic developers, from selection from pre-existing modules to device design from scratch, and that connectivity and interface design is in most cases not addressed properly (Panikowska et al., 2016). However, a more structured and methodic approach to microfluidic structures and modules' design, where microfluidics functions as a service or tool instead of the product, and interface design is taken into account from the start, can lead to a more successful integration in the chemical engineering and biotechnological market. Furthermore, the provision of microfluidic related services, such as add-ons to existing devices (facilitated in a modular approach), software support, device maintenance, collection and disposal of used devices or cartridges (in the case of disposable and/or medical systems), would significantly improve profits and customer retention (Panikowska et al., 2016).

In this respect, the solution to the low market spread might be found in the development of simpler devices which are easier to (inter)connect, whether in plug-and-play approaches (the so called modular microfluidics approach) or to already existing external analytical equipment. Changing the focus from finding the “killer application” (Blow, 2007), (Au et al., 2016), to designing and manufacturing more flexible devices in terms of connectivity and applicability (Morgan et al., 2016), will significantly expand the range of possible applications (Haber, 2006), (Mark et al., 2010), (Wu and Gu, 2011a) of microfluidic systems. A generic platform, capable of facilitating operation, as well as the integration of multiple unit operations and their associated fluid handling, would undoubtedly boost the microfluidic field's influence in the global market (Mark et al., 2010), (Sabourin et al., 2013) and its acceptance by non-microfluidic experts. It is important to note here that the wide-spread application of microfluidics is bound by the costs of the final device, the complexity of operation and the dependency on external equipment as previously mentioned (Sia and Kricka, 2008), (Sackmann et al., 2014), but also on the development of more adequate business models (Mukhopadhyay, 2007). Such models may involve the focus on a single and highly required application (the yet to be found “killer application”, offering a targeted answer to a specific question (Sackmann et al., 2014)), the focus on an application field (e.g. diagnostics (Erickson and Wilding, 1993), (Sharma et al., 2015)), the miniaturization of existing equipment (e.g. flow chemistry (Adamo et al., 2016)) or processes or the development of the microfluidic tools applied in academia and/or industry in different fields (Blow, 2007). In this sense, better data gathering and analysis tools can provide more insight on supplier/consumer chain management, market growth and business risk assessment, and in turn result in better market forecasts and prediction of technology acceptance. There is currently a lack of studies on the technology acceptance of microfluidics, which is for other technologies (e.g. smart-watches and wearable technologies (Kim and Shin, 2015)) helping to guide their development towards more marketable products (Turner et al., 2010), (Marangunić and Granić, 2015). These tools are also required to increase the commercialization potential of microfluidic devices (Gärtner, 2017). For example, the EU project “FlowMap” identified the main market drivers and key aspects of economic development within microfluidics by interviewing 150 experts in the field and was thus capable of providing a relevant guide towards more informed business decisions by microfluidic-based companies (Ducrée and Zengerle, 2004).

Some companies have already started commercializing microfluidic systems as tools that can be applied in academia or in research environments, thus following one of the possible business markets mentioned above. The ones mentioned here have also focused on the end-user, thus developing systems with a simplified use. Nanogen's (Nanogen, Inc., San Diego, US) electronic addressing technology (NanoChip® Electronic Microarray) allows capturing DNA probes in specific locations towards detection of single-base-pair differences, in a "blank slate" platform where the users can define their own assay (Huang et al., 2006). This system however, as other similar devices, requires a bulky benchtop workstation for microfluidic device operation, such as the use of robotic liquid handling instruments for liquid handling automation in microfluidic systems (Haber, 2006). Epigem (Redcar, United Kingdom) designs and fabricates devices for specific applications, while also providing their own strategy for fluidic connection and gaskets for reversible encapsulation, as well as embedded circuit layers. However, there is no standardization, since the systems are either application or client specific. Other commercial producers, such as Micronit Microtechnologies (Enschede, the Netherlands), Microfluidic ChipShop (Jena, Germany) and ThinXXS Microtechnology (Zweibrücken, Germany) also strive towards multiple application systems by having developed standard chips for certain unit operations (capillary electrophoresis, reactors, mixers, etc.) (Blow, 2007). They also manufacture chips with standardized sizes (for instance microscopy slides and microtiter plates) and microfluidic connections, providing even stages for easy fluidic connection. Connectivity between their own chips is facilitated, while the connectivity with chips from other manufacturers or developed in house by the end user is not easily achieved. Dolomite microfluidics (Royston, UK), which also provides several microfluidic chips, mostly for droplet, particle and emulsion generation, has embraced modular microfluidics and provides with the Telos® system a platform for straightforward process scale-up through parallelization (Han et al., 2017), (Microfluidics, 2017). In terms of connectivity, they have developed a specific approach based on standard sized tubing and connectors customized to dolomite platforms. However, again, the challenges related to easy connectivity to other chips or equipment have not been solved. Some academic groups have also tackled this issue of inter-chip connectivity with interesting approaches based on the LEGO® (Billund, Denmark) "plug and play" concept (Pepper et al., 2007), (Yuen, 2008), (Rhee and Burns, 2008), (Yuen et al., 2009), (Lim et al., 2014), (Morgan et al., 2016) or even using LEGO® components (Langelier et al., 2011), (Sabourin et al., 2013), (Vittayarukskul and Lee, 2017). There exists also a commercial prototyping system called The LabMatrix™ that provides a set of standard modular chips for molecular studies that can be assembled on a microfluidic breadboard. The set includes microvalves, syringe pumps, stereomicroscope, UV detection and NanoFlow flow cells (Zhou et al., 2003).

In terms of the fields where microfluidics has had or has the potential to have the highest impact, biomedicine, diagnostics, the chemical industry and biotechnology seem the most relevant.

Health and biomedical applications have been in the focus of many research efforts in microfluidics, especially for applications in point-of-care (POC) diagnostics which represent around a third of the microfluidics overall market (Gärtner, 2017). Thus, most microfluidic systems available on the market serve a diagnostic or health monitoring purpose. The

compactness of microfluidic devices, low reagent consumption, rapid result turn-around time, coupled with disposability, requirement of small sample volumes and the possibility for on-chip storage of the required reagents, is seen as the solution to the majority of the hurdles related with diagnostics and treatment in remote or poorly-equipped locations (Sia and Kricka, 2008). POC diagnostics are also the microfluidic platforms that have most to gain from the development of fully self-contained or standalone (including result analysis) systems (Boyd-Moss et al., 2016). The most successful example of a microfluidic-based POC device is the i-STAT™ Portable Clinical Analyzer system from Abbot diagnostics. It is a handheld device that performs quantification of biological parameters in blood, based on microfluidic disposable cartridges with integrated electrochemical sensors, each cartridge fabricated for the quantification of a specific parameter or a set of parameters (Erickson and Wilding, 1993). This device revolutionized POC diagnostics by significantly reducing the time between sampling and result to around 5 minutes, thus enabling a fast action from health professionals. Furthermore, by performing the analytics automatically with self-calibration procedures and removing the need for sample pre-treatment, it can be used reliably by non-trained personnel, removing the risk of operator error affecting data quality and widening the settings on which such device can be applied to emergency rooms, disaster areas, family doctor offices, field research, etc. (Erickson and Wilding, 1993). The range of parameters it can quantify has expanded from blood electrolytes and hematocrit in the 1990s (Erickson and Wilding, 1993), (Jacobs et al., 1993), pH, blood gases, lactate (Bingham et al., 1999), (Dascombe et al., 2007) and cardiac troponin I (Apple et al., 2004) in the 2000s to total β -human chorionic gonadotropin immunoassay (Sowder et al., 2015), activated clotting time and measurements from intraosseous samples in the 2010s (Veldhoen et al., 2014). The device has even been applied to animals, such as horses, dogs (Verwaerde et al., 2002), sharks (Harter et al., 2015) and cattle (Yildirim et al., 2015). By targeting a set of relevant parameters, the i-STAT found its “killer application”, but due to the flexibility of the sensing system (electrochemical sensors) it has been able to expand its range of applications (e.g. developing new cartridges). The portability, reliability, self-calibration procedures, internal error detection system (indicating low or too high sample volume or hardware fault), simple sample introduction method and low sample volume required together with the variety of parameters measurable (depending on the cartridge use) has rendered the i-STAT an invaluable tool in healthcare settings by meeting the needs for relative low price, fast and near patient testing in hospitals (Bingham et al., 1999), that also embodies all the desired characteristics of a POC microfluidic device. Other commercially available microfluidic-based FDA-approved POC diagnostics are presented in Sharma *et al.* (2015), such as The Piccolo® from Abaxis (also for blood chemistry), Simplexa from Focus Dx, Quest (for flu A/B detection from nasopharyngeal swabs) and the BD MAX™ GBS Assay IDI-Strep B assay from HandyLab, BD (for detection of Group B *Streptococcus* from vaginal and rectal swab samples) (Sharma et al., 2015).

The specific needs of POC testing, especially simplicity of use and minimal requirement for auxiliary equipment, meet in part the aim of a plug-and-play approach, but also pinpoint the downside of this approach: the dependence on fluid handling equipment and limited sample (pre-)treatment units (Sia and Kricka, 2008).

Modular microfluidics is not the ideal solution for POC diagnostics, where a single all-round disposable platform is preferable to several multi-purpose chips in order to simplify use (by non-trained personnel), reduce contamination risk, use of unprocessed specimens and allow long-term storage of reagents in non-refrigerated settings, enabling an automated analysis. However, modular microfluidics seems extremely relevant to most other applications of microfluidics. Multi-purpose devices, especially reusable ones, enable to: (i) lower the cost of the method(s) used; (ii) increase the diversity of tests that can be performed, as well as the diversity of samples that can be handled; and, (iii) probably allow for a higher degree of comparison between samples, since the same devices can be used even if assembled differently.

An extremely relevant market for modular microfluidics has been synthetic chemistry and the production of valuable chemicals (Hessel et al., 2013), such as positron emission tomography (PET) tracers, active pharmaceutical ingredients (API) (Chiu et al., 2017), natural products (Pastre et al., 2013), fine and bulk chemicals, particle synthesis, pigments (Elvira et al., 2013), among others. Microfluidics provides valuable advantages in chemistry such as improved selectivity and process safety, smaller footprint, acceleration of mass-transfer limited reactions, increase in production rates through a scale-out approach and the intrinsic continuous, rather than batch, production (Chiu et al., 2017). Hartman *et al.* (2011) provide a good analysis of flow chemistry vs. batch reactors (Hartman et al., 2011). Microfluidics, especially based on droplet generation, is enabling the production of new microparticles and nanomaterials (Chiu et al., 2017), but there are already examples of modular-based flow chemistry being applied to industrial sized production (Elvira et al., 2013). Kockmann *et al.* (2011) discuss one of the first examples of a modular microreactor applied to chemical production, the Lonza reactor (Kockmann et al., 2011). They observed that the plate approach enabled great versatility in terms of the types of reactions it was capable of carrying out. This occurred since plates with different sizes and or geometries could be combined as a plate stack reactor and thus be easily adapted to the constraints/conditions of the reaction in question (Kockmann et al., 2011). Moreover, Mardani *et al.* (2017) describe the development of a micro-plant from the separate characterization of the reactor and distillation modules to their joint application. Interestingly, they further tested the micro-plant in the case of failure to feed one of the substrates, and were capable of observing phenomena that are usually only visible at pilot scale (Mardani et al., 2017). Also, Adamo *et al.* (2016) presented a module-based continuous manufacturing platform capable of combining both synthesis and formulation of several pharmaceutical compounds. As proof-of-principle, they produced four different compounds at a gram-per-hour scale. The use of such flexible, reliable and compact manufacturing platforms could simplify formulation of compounds with short shelf-life as well as lower the price of pharmaceuticals for small patient populations (Adamo et al., 2016). They can also allow the simultaneous test of potentially new compounds by using several micro-plants to replicate the process conditions (Mardani et al., 2017). Modular based small production plants may also facilitate adaptation to a rapidly changing market, thus reducing investment risk, by considerably decreasing lead time from lab investigations to production scale, as well as reducing planning efforts (Buchholz, 2010), (Bramsiepe et al., 2014). As discussed by Bramsiepe *et al.* (2014) the impact of modular-based production plants is especially relevant when testing failure tolerances, as well as automation and control

strategies by enabling the use of similar equipment across all the process development scales (Bramsiepe et al., 2014). The same strategy can as well be applied for microfluidic-based modular plants, especially in the production of pharmaceuticals as previously exemplified by (Adamo et al., 2016).

The potential of modular platforms is currently applied to biotechnology by taking advantage of novel approaches to synthesis, developed in flow chemistry (Bolivar et al., 2011), (Wohlgemuth et al., 2015). The physical separation of the reactions in a multi-enzyme cascade system can allow a better control of the overall reaction, especially if the product acts as an inhibitor on the following reaction or promotes undesired side reactions (Gruber et al., 2017). Physical separation may also enable a better quantification of generated compounds (Bi et al., 2015), as well as the separate characterization of the different enzymes involved in a metabolic pathway (Kampe et al., 2014). Furthermore, connection of the reactor modules with chiral columns, solvent extraction modules, filtration and separation systems, allows the exchange of solvents or buffers between reactor modules, as well as removing enzymes and/or products that might affect the next reaction in the sequence, and thus greatly improve the overall yield and productivity (Asanomi et al., 2011), (Gruber et al., 2017), (Žnidaršič-Plazl, 2017). By using a modular approach, a complex reaction system can be more rapidly optimized and downstream sample processing easily modified if a new product becomes more interesting. Furthermore, a similar module-based scale-up strategy to the one presented by Han *et al.* (2017) for functional materials (Han et al., 2017), could be used to increase productivity. The use of modules, each containing either a few channels or a vertical stack of microfluidic chips, also seems to be a viable solution to imbalances in fluid distribution in scale-out approaches (Wang et al., 2014), (Han et al., 2017). Žnidaršič-Plazl (2017) presents some recent examples of the applications of modular microfluidics for industrially relevant biotransformations and biocatalytic reactions, as well as an interesting perspective on the contributions of microfluidics to the biotechnological field (Žnidaršič-Plazl, 2017).

Another relevant example of the potential of modular platforms is the “body-on-a-chip” or “human-on-a-chip” concept. Here, each organ or tissue relevant to the physiological function being studied, or in the drug metabolism being tested, is mimicked in a microfluidic system and interconnected for an overview of the human biological mechanisms underlying the targeted morphogenetic and/or pathogenetic process (Perestrelo et al., 2015). The separation of the physiological systems in different chips allows for the use of optimized conditions for the on-chip differentiation or maturation of the different cell types during organ and/or tissue growth in the microfluidic platform (Loskill et al., 2015). It further allows minimizing the effect of variability between cell batches and cell lines on the overall human-on-a-chip observed when the several cell compartments are permanently connected (Loskill et al., 2015). The use of the modular human-on-a-chip approach also enables, by adding redundant single organ units, to bypass malfunctioning units without losing the multi-organ functionality (Loskill et al., 2015). Huh *et al.* (2013) developed a microfluidic chip for tissue-tissue interaction in organ-on-a-chip applications which can be used to grow and mimic different human tissues (e.g. lung alveoli, intestinal wall, kidney glomerulus) (Huh et al., 2013). The ATHENA (Advanced Tissue-engineered Human Ectypal Network Analyzer) platform, or “*Homo Minutus*”, is a project that aims to interconnect, through an artificial circulatory system, four human organ constructs (liver, heart, lung and kidney) (Dance, 2015). Loskill *et*

al (2015) (Loskill et al., 2015) developed the μ Organo, a customizable Lego®-based modular multi-organ system, that enables initial cell culture in individual organ chips, followed by their interconnection to achieve a microphysiological multi-organ system (Loskill et al., 2015). The combination between different organs is in this case performed with a toolbox of simple connectors (Loskill et al., 2015). Another project, the HeLiVa platform, aims at integrating three organ constructs (heart, liver and vascular systems) on a chip with the necessary assay, labelling and analytical procedures for detailed analysis (Vunjak-Novakovic et al., 2013). And recently, Edington *et al.* (2018) have developed a microfluidic system that integrates up to 10 microphysiological systems in a single chip (Edington et al., 2018). Such “human-on-a-chip” systems can accelerate drug development by enabling trials in human tissues, as well as facilitate the study of certain diseases (e.g. cancer). Moreover, *InSphero AG* is a Swiss company developing organ models based on cellular spheroids floating in wells and connected by microchannels. They commercialize liver, pancreas, tumour and skin 3D cell cultures, already prepared for *in vitro* toxicology and drug discovery assays, as well as assay kits (Perestrelo et al., 2015), (InSphero, 2017).

Figure 1 shows examples of some of the mentioned applications of modular microfluidics, such as the chemical industry (e.g. production of APIs), biotechnology (e.g. biocatalytic process) and “body-on-a-chip” (e.g. “heart-on-a-chip”).

Figure 1 – Examples of microfluidic platforms and applications of microfluidics: (a) modular-based biocatalytic process integrating a cascade reaction with in-situ substrate supply and product removal (Gruber et al., 2017) (image reproduced from Gruber et al. 2017 with permission from Biotechnology Journal); (b) modular-based continuous production (“A”) and downstream process (“B”) of APIs (Adamo et al., 2016); (c) modular-based microfluidics for assembly of “body-on-a-chip” representing in “a” general procedure of culture and assembly of such device, and in “b” and “c” staining of cultured cells and beating motion of the formed cardiac tissue (Loskill et al., 2015) (image reproduced from Loskill *et al.* 2015 with permission of PLoS ONE);

Despite such tremendous advances in microfluidic technology and its applications, most of the major developments in the microfluidics field foreseen in 2004 by Erickson and Li (Erickson and Li, 2004), such as decreased dependence on external equipment towards higher portability, and an increased use of simulation and modelling for device design optimization in the initial stages of device development, still remain to be achieved. Even though a decreased reliance on external equipment was obtained for some applications, most systems still rely on external pumps, potentiostats, manual/automatic external sample pre-treatment, microscopes, power sources, etc. The integration of light sources in a planar format, such as organic light-emitting diodes (OLEDs) (Lefèvre et al., 2015), is the example of an approach towards a decreased reliance on auxiliary equipment through its miniaturization and integration.

Also, a higher number of fabricated microfluidic devices are currently being studied by means of mathematical and numerical tools, such as Matlab® (Schäpper et al., 2011) and computational fluid dynamics (CFD) (Rosinha Grundtvig et al., 2017), with the aim of reducing development time. These software tools have contributed to a considerable progress in recent years in accommodating the faced challenges when modelling at this scale. However, the lack of standard analytical tools at microscale often hampers the experimental validation of the numerically predicted phenomena. Thus, without proper experimental validation, it is

often difficult to convince the user of microfluidic devices of the legitimacy of simulation results. An example of such experimental validation can be found in Hoffman *et al* (2018) (Hoffmann et al., 2018).

2. The challenge of integration

As discussed in the previous section, the market presence of microfluidic or microstructured devices is still limited. This limitation is partly related to poor acceptance of such devices by the final users (usually non-microfluidic experts), their limited range of applications (focus on a specific target application) and the cost of their introduction as the new standard in a given field. Easily exchangeable and interconnectable modules might resolve these constraints. The challenge of achieving such modules thus relies on their integration and use as a single platform.

The integration of several unit operations at microscale was initiated together with the microfluidic fabrication field at the end of the 1980's (e.g. fluid displacement (van Lintel et al., 1988), sensing and separation (Manz et al., 1990), (Manz et al., 1992), (Mark et al., 2010)). The semiconductor industry investigated the development of monolithic miniaturized components called micro-electro-mechanical systems (MEMS), such as sensors, valves, separators and mixers, due to the discovery of a suitable material, silicon (Petersen, 1982). The development of MEMS systems was further enabled by the outsourcing of the semiconductor production to the upcoming Asian countries, which allowed to free the existing semiconductor production capacities for research purposes (Bryzek, 1996). Miniaturized components based on polymeric materials were also developed, with the appearance of soft-lithography methods (Duffy et al., 1998), (Duffy et al., 1999). The coupling of several of these components proved however to be technically complicated. The main reason behind this was the choice in fabrication technologies of the individual components, which were usually incompatible (Mark et al., 2010), as well as the design of both the channels with different dimensions (leading for example to disparate required flow rates for different components) and connections between platforms (mostly un-standardized). Therefore, simpler approaches, applying bench scale equipment (external pumps, microscopes, etc.) were pursued to facilitate the development of individual unit operations (Wu and Gu, 2011b). Consequently, in order to reduce the dependency on external devices, the effort towards the development of standalone microfabricated devices has recently increased. Boyd-Moss and co-workers have recently published an excellent review on standalone or self-contained microfluidic systems for biomedical diagnostics, where a thorough analysis of their main concepts, operating mechanism (passive, such as capillary action; hand-powered, such as by pulling a syringe; or active) and output is performed (Boyd-Moss et al., 2016). The constant miniaturization of electronic components is mentioned as a relevant driver in achieving self-contained operation (e.g. portable power supply, piezoelectric pumps) and sorting (e.g. dielectro- and acousto-phoresis) (Boyd-Moss et al., 2016). A standalone device is one type of microfluidic system or platform.

A microfluidic platform consists of a set of microfluidic elements, each previously and individually validated, capable of performing a given fluid handling or sample

treatment/measuring step (unit operation). Ideally, these unit operations should be capable of being combined and assembled differently depending on the final application (Mark et al., 2010). The integration of various unit operations on a single device requires a holistic understanding of the characteristics of the substrate materials, the available or possible fabrication technologies, the characteristics of the target sample, the devices's final application and the environment in which it will be applied.

The material(s) composition of a microfluidic system is chosen according to the platform's required function, degree of integration and application (Nge et al., 2013). Characteristics such as air permeability, biocompatibility, nonspecific adsorption, surface functionalization, optical transparency, flexibility, solvent compatibility, electrical compatibility, and opportunity for sterilization are considered when choosing a material for a specific application. The robust and leakage free integration of different materials in the same platform is a further challenge (Mariella, 2008). The most frequently used materials can be divided in three categories: inorganic, polymeric, and paper. A summary of their characteristics is presented in Table 2.

The selection of the fabrication technique is dependent on the choice of materials, the final application of the device, how robust it needs to be and whether or not reusability is required. The choice is also dependent on the type of end user (experienced or not), location of use (point-of-care vs. research laboratory, for example), and the time between fabrication and use (is storage required?). Material properties also influence the minimum attainable feature dimension (Becker, H., Beckert, E., Gärtner, 2009). These characteristics will guide the choice of the fabrication methods, from a wide variety available for the production of microfluidic devices. These include prototyping techniques (such as hot embossing, injection molding and soft lithography) and direct fabrication techniques (such as thin film deposition, laser photoablation, photolithography/optical lithography, etching and 3D printing). Furthermore, the choice of a fabrication method takes into account the desired minimum feature dimensions, surface roughness and aspect ratio of the channels, as well as the tolerances and reproducibility of the method, the selected chip material and the final application. For more complex applications, compatibility between different fabrication methods should also be considered during the selection. Hecke et al. (2003) (Hecke and Schomburg, 2003), Ziaie et al. (2004) (Ziaie et al., 2004), Becker and Gärtner (2008) (Becker and Gärtner, 2008), Wu and Gu (2011) (Wu and Gu, 2011a), Iliescu et al. (2012) (Iliescu et al., 2012), Cheng et al. (2012) (Cheng et al., 2012), Li, et al. (2012) (Li et al., 2012), Ho et al. (2015) (Ho et al., 2015) and Au et al. (2016) (Au et al., 2016) present a good overview of past and current fabrication techniques, and also discuss the main considerations for the selection of the different methods.

Along with the choice of the material and the fabrication technique, the type of microfluidic platform needs to be considered in advance, such as: single or multiple unit operations on the same chip; single platform or part of a platform; integrated quantification and fluidic handling or connection with external equipment; and, importantly, also how to perform data and signal acquisition and treatment. When considering connectivity of the microfluidic chip, several other aspects should also be taken into account: (i) disposability of the device; (ii) to which devices it will connect; (iii) inlets positioned in-plane or perpendicular to the chip; (iv) should it be application-specific; (v) area occupied by connections; (vi) fabrication process; (vii) pressure and temperature tolerance; (viii) compatibility between

materials used (e.g. solvents as target detection solutions); (ix) dead volume generated in the connections; (x) sterility; (xi) permeability; (xii) type of sample; and, (xiii) price (Iliescu et al., 2012). Some of the aspects mentioned were also presented in the material and fabrication selection section above. However, it is relevant to mention them again since their selection may be different depending on whether inter-chip connectivity is considered or not. Microfluidic interconnections need to provide a low pressure drop and dead volume and hermetic seal, coupled with a reliable performance under multiple uses (Perozziello et al., 2008). Manually fabricated connections may not be built reproducibly, and are thus adding variability to the flow or the operation of the system. The adoption of a standard size inlet diameter, that allows the use of finger tight fittings and standard tubing, facilitates interconnection with other platforms using the same type of connectors, as well as connectivity to most external equipment (HPLC devices and mass spectrometers (Kirby and Wheeler, 2013) or Raman spectrophotometers (Perozziello et al., 2016) as well as syringe pumps).

However, as stated in Wu and Gu (2011) (Wu and Gu, 2011b) microfluidic platforms are usually developed with a specific or a small range of applications in mind. This often requires a re-design when a modification, addition of another function or integration with other systems or platforms is needed. In Hlawatsch *et al* (2012) (Hlawatsch et al., 2012), different process modules, previously optimized for a target application (Gartner and Becker, 2008), were left separated and used in series (“plug-and-play”) in order to provide more flexibility in terms of application of the final microfluidic setup. For each application, different modules or the same modules, but in a different order, can be used. Thus, the development of unit operation modules can widen the applicability of a microfluidic system, as well as reduce the time spent in designing and optimizing a system for a given application.

Figure 2 presents a simplified representation of the different modes of use and approach to microfluidics mentioned thus far: standalone vs. dependent on external equipment, and multi-unit operations in a single chip vs. “plug-and-play” approach.

Figure 2 – Simplified representation of different types of microfluidic systems or platforms: (a) standalone platform (that contains all the components required to carry out the target process or assay) exemplified by the i STAT™ Portable Clinical Analyzer system; (b) standard microfluidic system (that depends on certain external equipment such as pumps and analytical devices to carry out the target process or assay); (c) Conventional approach to microfluidic platforms (miniaturization and integration of all the necessary unit operations in the same chip), exemplified by an image taken from Boyd-Moss et al. (2016) (Boyd-Moss et al., 2016) with permission of the Royal Society of Chemistry; and (d) modular approach to microfluidic platforms (integration of all the required unit operations as separate miniaturized unit operations that are interchangeable and replaceable by new units if needed).

2.1 Available unit operations in microfluidic chips

To guarantee a wide applicability of modular microfluidic platforms it is essential that the relevant unit operations to most applications are available, and exist or can rapidly be made in such a format. In general, a “plug-and-play” system should contain the following elements:

- **Fluid handling unit:** Such a unit could function as a pump, allowing appropriate flow of the sample in the system, with good control of flowrate and type of flow, ideally allowing a range of possible flow velocities. Another important fluid handling

function is a valve system, especially a multi-port valve that enables the control of the entrance, mixing or even the path inside the system of different fluids. Sabourin *et al* (2013) (Sabourin et al., 2013) developed a very interesting system where liquid handling is automatically achieved with miniaturized and integrated pumps. Other groups decided on a simpler approach such as a magnetically actuated stirrer-based micropump (Kimura et al., 2015), valves actuated by tightening a screw (Zheng et al., 2009) or using a Braille display (Gu et al., 2007), or even capillary forces (Madadi et al., 2015). Oh *et al.* (2012) presented an interesting guide on design of microfluidic networks to ease fluid handling (Oh et al., 2012). Electroosmotic flow (EOF) offers an interesting alternative to pressure driven flow, where the flow front has a flat profile, being capable of generating high flowrates without moving parts (Gao and Gui, 2016). It has been widely applied in bioassays, drug delivery, fuel cells, sludge treatment and microelectronic chip cooling (Gao and Gui, 2016), (Lim et al., 2017). Passive approaches to valves can also use capillary bursts, and stimuli-responsive hydrogels (Wang et al., 2005) (Boyd-Moss et al., 2016). It is also relevant to develop integrated systems that are able to validate and measure the generated flow inside microfluidic networks, as well as being able to determine backpressure. A simple system, based on luminescent optical sensors, as presented by Hoera *et al.* (2017), is an attractive possibility (Hoera et al., 2017) and enables the simultaneous measurement of temperature and oxygen concentration.

Figure 3 – Braille display developed by Gu *et al.* (2007) as a fluid handling unit. Figure adapted from (Gu et al., 2007).

- **Mixing/dilution unit:** Mixing is an extremely important function when performing reactions or studying the influence of certain compounds, since it needs to occur faster than the reaction effect being studied in order not to significantly influence the outcome (Liau et al., 2005). At microscale, mixing occurs mainly through diffusion, but certain strategies can be applied to improve mixing efficiency. Significant mixing strategies involve passive approaches which are based on the generation of chaotic mixing with channel bends or topology in the channel (Liau et al., 2005), or on increasing the contact area between samples via lamination or intersecting channels (Lee et al., 2011). A novel approach to passive control over mixing uses stimuli-responsive hydrogels (Prettyman and Eddington, 2011). Diverse active mixing strategies, such as acoustically-induced microstreams, dielectrophoretic micromixers, electrokinetic actuation (Gao and Gui, 2016), velocity pulsing and magneto-hydrodynamic flow have also been thoroughly developed and applied (Lee et al., 2011).

Mixing can also be performed in order to achieve gradients of certain components through dilution. Niu *et al* (2011) developed a droplet-based platform capable of performing dilutions within a range of four orders of magnitude by splitting and (re)merging droplets to create reagent gradients (Niu et al., 2011). Rho *et al* (2016) used peristaltic mixing in controlled volume microreactors to generate stepwise concentration gradients of two reagents (Rho et al., 2016). The induction of

convective mixing is also very relevant in liquid-liquid extraction, especially in slug, droplet and dispersed flow (for details, see (Kurt et al., 2016)).

- **Sample concentration unit:** This is especially critical for applications that involve extremely diluted samples (Mariella, 2008), which can range from water quality testing to detection of cancer cells or viruses in blood. Also, in the human body the physiological concentration of considerable compounds is in the order of nM or lower, thus requiring pre-concentration units for allowing detection. Several strategies involve adhesion of the molecules or cells to the channel walls, which can be functionalized (Stott et al., 2010) or not (Jing et al., 2013), using chaotic flow induction to increase enrichment performance. Recently, Pereiro *et al.* (2017) developed a fluidized bed microreactor capable of capturing bacteria from liquid raw samples (e.g. milk) using functionalized magnetic beads (Pereiro et al., 2017).

Figure 4 - Fluidized bed microreactor developed by Pereiro *et al.* (2017) (Pereiro et al., 2017). The figure presents the magnetic field gradient with magnetic forced oriented towards the chamber inlet (a) and the influence of flow on the packed magnetic beads (b). Image reprinted with permission from I. Pereiro, A. Bendali, S. Tabnaoui, L. Alexandre, J. Srbova, Z. Bilkova, S. Deegan, L. Joshi, J.-L. Viovy, L. Malaquin, B. Dupuy, S. Descroix, A new microfluidic approach for the one-step capture, amplification and label-free quantification of bacteria from raw samples, *Chem. Sci.* 8 (2017) 1329–1336. doi:10.1039/C6SC03880H – Published by The Royal Society of Chemistry.

- **Filtration/ purification unit:** Units capable of removing contaminants, or separating cell debris or types of cells from the sample are highly valuable as sample treatment units. Strategies applied to sample filtration/ purification use differences in (i) size (e.g. using capillary forces in a microchannel integrated micropillars (MIMPs) chip to separate plasma from blood (Madadi et al., 2015) or simultaneous isolation of multiple antibodies from serum and multiple cell types from blood using microbeads (Sarkar et al., 2016)), (ii) functionalization of channel surface (e.g. with avidin and treated with antibodies conjugated with biotinylated photocleavable crosslinkers with a specific 19-mer DNA sequence to capture cancer biomarkers directly from whole blood (Stern et al., 2010)), (iii) immunomagnetic separation (e.g. immunomagnetic beads and a micro-aperture chip to separate circulating tumour cells (CTC) from whole blood samples (Chang et al., 2015)), (iv) adhesion to silica (e.g. extraction of RNA from prepared rat tissue samples using a porous silica monolith column (Shaw et al., 2013)), and (v) solid-phase extraction (e.g. using cation exchange resins (Park et al., 2015)).

Concentration and filtration/purification units often function as the same unit, since by isolating or separating a target cell/particle/molecule, its concentration from the initial sample is achieved. In chemical engineering, separators are often more relevant than filtration, for downstream concentration/ purification of the target compound. Separators allow the recovery of the target compound after liquid-liquid extraction. Gürsel *et al.* (2017) present a good overview of current modular microfluidic approaches both to liquid-liquid extraction (usually mixers for immiscible liquids) and separation units, focusing in the latter case on counter-current-flow approaches. Some of the separator modules described present similar approaches to the filtration / purification units described above (e.g. surface treatment, membranes, micropillars). Gürsel *et al.* (2017) further discuss in detail the impact of the type of flow regime (parallel, slug and dispersed flow) on extraction performance and simplicity of separation

operation. They also provide an interesting perspective on the role microfluidics plays in achieving end-to-end continuous manufacturing (Gürsel et al., 2017).

- **Sorting unit:** Besides the ability to isolate a target molecule or cell from a complex sample, it might also be required to differentiate among the purified molecules or cells for a certain characteristic, for which sorters can be used. This is especially relevant when establishing protein or genetic libraries or developing mutants. Sorting of molecules, cells, particles or droplets can be performed using electrostatic actuation (such as, dielectrophoresis (Niu et al., 2007) (Adam R Abate et al., 2010) (Frenzel and Merten, 2017) or electrostatic charging (Link et al., 2006)), optical approaches (such as optical tweezers or traps (Wang et al., 2011) or fluorescent activated cell sorting (FACS)), mechanical approaches (e.g. with membrane valves (Adam R. Abate et al., 2010)), acoustic approaches (e.g. surface acoustic waves (Wang and Zhe, 2011)), magnetic approaches (e.g. magnetophoresis or magnetic activated cell sorting (MACS)), channel topography (Hsu et al., 2008), inertial or hydrodynamic focusing or affinity approaches (Z. T. F. Yu et al., 2014).
- **Sample amplification:** Such a unit may enable working around the issue of highly diluted samples, allowing to replicate the target molecules (e.g. DNA or mRNA) (Mariella, 2008). However, the issue of retaining or capturing such molecules remains. Most amplification units perform polymerase chain reaction (PCR) either in chambers (e.g. reverse transcription PCR (RT-PCR) using a thermoelectric Peltier element for temperature control during amplification (Shaw et al., 2013)) or continuously in channels (e.g. real-time PCR of single-DNA per droplet in a circular channel design with zones at different temperatures (Schaerli et al., 2009)). Other amplification techniques such as multiple annealing and looping-based amplification cycles (MALBAC) (Z. Yu et al., 2014) and nucleic acid sequence-based amplification (NASBA) (Dimov et al., 2008) have also been miniaturized in microfluidic devices.

Figure 5 - Multiple annealing and looping-based amplification cycles (MALBAC) developed by Yu *et al.* (2014) (Z. Yu et al., 2014). The figure shows the schematics of the device (a) with the microfluidic channels in purple and the control channels in magenta, the different MALBAC reactions (b), the thermocycler used for temperature control (c) and the scattering of a single cell on the chip (d). Image reprinted with permission from Z. Yu, S. Lu, Y. Huang, Microfluidic Whole Genome Amplification Device for Single Cell Sequencing, *Anal. Chem.* 86 (2014) 9386–9390. doi:10.1021/ac5032176. Copyright 2014 American Chemical Society.

- **Incubation unit:** Such a unit can either work as a reactor, allowing for a certain reaction to occur for a defined residence time, a labelling unit, or even as an incubation chamber, allowing for growth of organisms. This type of unit requires an excellent control of volume and residence time, and has been extensively used together with droplet microfluidics or single-cell platforms. Several droplet microfluidic platforms present incubation units, which are chambers where the cells or droplets are stored (Mary et al., 2011), (Theberge et al., 2012) or long channels that allow for a tight control of incubation (residence) time (Adam R Abate et al., 2010).

Strategies for other sample pre-treatment units are presented by de Mello and Beard (2003) (e.g. liquid-liquid and solid-phase extraction, isotachophoresis, cell lysis) (de Mello and Beard, 2003), Chen and Cui (2009) (e.g. dielectrophoresis, magnetic activated cell separation,

DNA purification) (Chen and Cui, 2009) and Huang *et al* (2002) (e.g. nucleic acid amplification and purification, microfiltration) (Huang et al., 2002).

- **Detection unit:** Quantitation of target compounds is one of the major functions and advantages of microfluidics, due to the variety of sensors available, which offers a possibility for real-time and continuous monitoring and proximity to the samples. An overview of the different types of available sensors for microfluidic applications is presented later in the text, but the variety of available sensors ranges from electrical (such as, dielectric determination of size, shape and composition of droplets at high speed (Niu et al., 2007), or following changes in cell size, capacitance and liquid exchange by electrical impedance spectroscopy (Bürgel et al., 2015)) to optical (e.g. recent application of stroboscopic epifluorescence imaging to hundreds of droplets simultaneously (Hess et al., 2015), surface-enhanced Raman scattering (SERS) detection of hazardous materials (Quang et al., 2008), the use of Fourier Transform Infrared (FT-IR) microscopy in studying enzyme kinetics (Polshin et al., 2014), or the application of optoelectronic devices, such as organic photodetectors (Lefèvre et al., 2015)) and even nano-wires (e.g. nanoribbons capable of performing the detection of multiple biomarkers simultaneously (Stern et al., 2010)). An effort towards the development of multiple application sensors or sensors capable of measuring more than one component or parameter simultaneously would also greatly contribute towards more flexible modular systems. The integration of the sensing units in the microfluidic platform is a major step in microfluidic design and different approaches have been used, from irreversible integration (e.g. sensor layer deposition prior to sealing channel (Nirschl et al., 2011), (Fernandes et al., 2014), (Lefèvre et al., 2015), during channel fabrication (Yuen, 2016a), (Gaal et al., 2017) or after channel sealing (Frey et al., 2010)) to reversible integration (e.g. through threaded ports (Erkal et al., 2014) or with chip holders (Tkachenko et al., 2009), (Wilhelm et al., 2013)).

Figure 6 – On-chip electroporation and impedance spectroscopy device developed by Bürgel *et al.* (2015) (Bürgel et al., 2015). The figure shows a cross-sectional schematic of the device's active area, showing both the impedance spectroscopy measurement and cell electroporation after measurement (a) and a photograph of the microfluidic chip (b).

The presented list of existing microfluidic chips (summarized in **Table 1**), capable of performing the most relevant and essential unit operations in any screening, optimization or development study is not exhaustive. Boyd-Moss *et al.* (2016) present a more complete list of the common mechanisms used in microfluidic systems to achieve flow driving and control, mixing, sorting, amplification and target detection (Boyd-Moss et al., 2016). They also highlight the dependence of some of these mechanisms on external equipment. There is a multitude of approaches for solving pre-treatment and sample concentration issues for example, that with little or no modification could be coupled together and/or integrated in a modular platform (e.g. on-chip single cell electroporation (Bürgel et al., 2015)). The common approach however, especially towards achieving standalone systems, is the integration of all the unit operations on the same chip to simplify usage and guarantee accuracy of the data (Boyd-Moss et al., 2016).

Notwithstanding, we believe that by combining the different presented microfluidic systems, most processes in the biotechnological and health fields could be studied on-chip in a flexible “plug-and-play” approach.

Table 1 – Summary of the presented unit operations currently available for microfluidic chips.

2.2 A perspective on modular microfluidics

A modular approach to microfluidics provides specific advantages, such as the increased operation flexibility by facilitating the microchannel or microfluidic system reconfiguration, where a certain unit operation can be easily substituted or the order of different unit operations in a process can be changed (Bhargava et al., 2014). Furthermore, by using connected discrete components, the same unit operations can be applied to different analytical, reaction or downstream purposes, thereby lowering the cost of the final setup. Additionally, by re-using the discrete components, the assembly and planning of different setups is facilitated, since each system can be previously characterized (Bhargava et al., 2014).

A wide application of microfluidics can be achieved if not only modularization, but also standardization of connectors is achieved. Connectivity between the above units could be attained with some of the connectors and interconnectivity ports presented in Figure 7, such as the one from Pepper *et al.* (2007) (Pepper et al., 2007) (Figure 7 (a)), where “click on” connectors to standard tubing sizes were developed, and Sabourin *et al.* (2013) (Sabourin et al., 2013) (Figure 7 (b)), who developed multi-connector ports. Connectivity to electrical interfaces could also be carried out as presented in Yuen *et al.* (2008) (Yuen, 2008) (Figure 7 (c)), who based their design of fluidic and electrical connections on an electrical breadboard. Yuen (2016) has also developed stick-and-play connectors using magnets (Yuen, 2016b).

Figure 7 – Schematics of the connectivity approaches presented by Pepper *et al.* (2007) (Pepper et al., 2007) (a), Sabourin *et al.* (2013) (Sabourin et al., 2013) (b) and Yuen *et al.* (2008) (Yuen, 2008) (c). Images adapted from: (a) Pepper, M., Palsandram, N.S., Zhang, P., Lee, M., Cho, H.J., 2007. Interconnecting fluidic packages and interfaces for micromachined sensors. *Sensors Actuators, A Phys.* 134, 278–285; (b) Sabourin, D., Skafte-Pedersen, P., Søe, M.J., Hemmingsen, M., Alberti, M., Coman, V., Petersen, J., Emnéus, J., Kutter, J.P., Snakenborg, D., Jørgensen, F., Clausen, C., Holmstrøm, K., Dufva, M., 2013. The MainSTREAM component platform: a holistic approach to microfluidic system design. *J. Lab. Autom.* 18, 212–228; (c) Yuen, P.K., 2008. “SmartBuild-a truly plug-n-play modular microfluidic system”. *Lab Chip* 8, 1374–1378

Adaptation of the units presented in section 2.1 to the building block concept introduced by Rhee and Burns (2008) (Rhee and Burns, 2008), Langelier *et al.* (2011) (Langelier et al., 2011) and Vittayarukskul and Lee (2017) (Vittayarukskul and Lee, 2017) would further increase the flexibility and potential of the modules as part of a multi-use “plug-and-play” platform. Figure 8 presents another example of a modular microfluidic concept [Bhargava, *et al.* (2015) (Bhargava et al., 2015)], based on 3D printed modules, which is compatible with a non-planar microfluidic circuit assembly (Bhargava et al., 2014). Another 3D printed modular-based microfluidic platform has been developed by Morgan *et al.* (2016) (Morgan et al., 2016).

Figure 8 – Example of what a modular-based microfluidic device may look like. The figure, from Bhargava, K. C. *et al.* (2015) (Bhargava et al., 2015), presents a circuit diagram (a) of a hydraulic circuit with two inputs (b) with syringe attached (c) for withdrawing fluids. Image used with permission from (Bhargava et al., 2015) under a Creative Commons Attribution 4.0 International License.

A set of mass-produced building blocks that can be arranged in a multitude of different channels and even 3D shapes could be acquired by every research institute, company or diagnostics center, for easy assembly towards their target research. The building blocks could be available together with a simple set of miniaturized electronic components (e.g. pump, charge-coupled device (CCD) imaging technology, potentiostat, etc.) that function both as part of the fluidic and detection units, but also as user-friendly validation units of the assembly. An example of a miniaturized detection system for such a toolbox is the reconfigurable Photonic lab on a chip (PhLoC) developed by Ackermann *et al.* (2016) (Ackermann *et al.*, 2016). A true easy-assembly microfluidic toolbox could thus be accessible to everyone, allowing for a widespread use of microfluidics, much like Raspberry Pi or Arduino has taken individual programming and building of electric circuits to a new level. Another approach to the development of such microfluidic toolbox could involve the use of home-made or economically accessible three-dimensional printing devices, coupled with a database of designs of microfluidic platforms. Such database could be developed as an extra effort towards the standardization of the designs of microfluidic modules (Erkal *et al.*, 2014), similar to current attempts at sharing and/or facilitating access to developed mathematical and mechanistic models (Kent, 2002), (Argent, 2004), (Wolkenhauer *et al.*, 2014), (King *et al.*, 2016). For the latter, this has resulted in effortless incorporation and application of different models, even when developed in different computational languages. The use of 3D printing technology, due to its simplicity of use, fast replication of intricate designs and variety of available materials would increase microfluidics accessibility to non-experts (Gaal *et al.*, 2017). Furthermore, the fast replication abilities would enable a short design-to-product time, accelerating the development and improvement of existing modular fluidic and/or connection designs, or even promoting the rapid testing and sharing of new designs (Gaal *et al.*, 2017). A modular approach, in general, facilitates the substitution of any given part towards the optimization of the overall process without disrupting the entire setup or altering too much the system characterization performed thus far (Bhargava *et al.*, 2014). Figure 9 presents a more detailed overview of the two paths towards standardized modular microfluidic systems that have been discussed.

Furthermore, as already proven for flow chemistry and small-scale chemical production, the use of modules and their selection from a module database, results in a significant lead time reduction (Bramsiepe *et al.*, 2014). Two European projects, F3 Factory (Buchholz, 2010) and CoPIRIDE (Löb, 2013) were especially relevant in proving the usefulness of modular micro-structured equipment to process intensification and reduction of development time in the chemical industry (Bramsiepe *et al.*, 2014). F3 Factory has resulted in the adaptation of several industrial batch processes to continuous production through application of modular micro-structured technology, with examples that can be consulted on the project's website (www.f3factory.com) and in (Bieringer *et al.*, 2016). A very detailed discussion on the role that modularization (at all scales) will have in "Industry 4.0", by increasing production plant flexibility in terms of capacity and type of feedstock, as well as shorter delivery and development types, is also presented in (Bieringer *et al.*, 2016). In the white paper by Bieringer *et al.* (2016) the main challenges related to such an approach to continuous manufacturing are also discussed (e.g. in terms of logistics and regulation standards), and possible business models for its market integration (e.g. rental equipment, special

maintenance services, remote operation of small-scale plants or production at the customer site) are presented (Bieringer et al., 2016). Even though not all the conclusions presented by Bieringer *et al.* (2016) can be applied to microfluidic-based modules, they offer a good guidance for the considerations to be made when commercializing microfluidic modules and micro-plants.

Figure 9 – Summary of envisioned approaches for the use of standardized microfluidic modules and modular platforms.

However, unlike mathematical models, the outcome and combination of microfluidic designs can vary greatly with the fabrication technique, especially its resolution, and/or materials available. Hence, careful selection of the fabrication technology and assembly method has to be made in order to ease device validation and modular application. Krtischil *et al.* (2013) present some novel mass-production approaches for modular microstructured reactors for production scale, such as roll embossing technique (Krtischil et al., 2013).

It is also important to consider that when selecting a module from a database such as the one described above, the system selected will be able to operate at the desired process conditions, but may not be the best system for the case-study in mind, as was also discussed in more detail by Bieringer *et al.* (2016) (Bieringer et al., 2016). For applications that require a higher degree of validation, such as biomedical applications, more complete individual unit blocks could be used. In such blocks, as previously mentioned, each building block would be validated separately, assuring leakage free and robust assembly and performance by using one of the connection strategies described above. The concept of an easily assembled, standalone “plug-and-play” microfluidic device for multiple applications will thus be made possible in the near future.

A concern in the use of a modular approach is the accumulation of fluidic resistance with each module that is added, which can affect flow (and thus downstream) performance and lead to the loss of reagents or involved particles/cells (Chiu et al., 2017). The achievement of consistent and reliable assembly and disassembly of the modules is a significant concern (Yuen, 2016b), which the connectivity approaches presented previously try to address and solve. In addition, depending on the chosen connectivity strategy, large dead volumes may occur between the modules and affect system performance, especially concentration and detection operations, but lead as well to a significant increase in pressure drop (Chiu et al., 2017). However, there are several strategies available to reduce pressure drops (e.g. division of flow in different channels (Jensen, 2001)) and fluidic resistance (e.g. surface modification, towards high hydrophilicity). Furthermore, as mentioned previously, flow uniformity in parallel channels can be controlled using serpentine structures as fluid resistors, positioned before the chip’s active area (Wang et al., 2014), (Han et al., 2017). Likewise, diverse flow generating strategies (e.g. electroosmotic or capillary flow) could be applied in combination with pressure-driven flow. Additionally, modules to control backpressure in the pumps, or allowing pressure equalization along the module assembly can be integrated. Moreover, as demonstrated by Bhargava *et al.* (2014) (Bhargava et al., 2014) and Bhargava *et al.* (2015) (Bhargava et al., 2015), for most current applications in microfluidics (incompressible flows

and low Reynolds numbers), the hydraulic characteristics (especially pressure loss and obtained flow rates) of a given system can be estimated using the Kirchoff's Laws which are usually applied to electronic circuits. Coupling the electronic circuit analogy with statistical analysis methods, different fluidic networks were simulated where an expected manufacturing variation from design (calculated from knowledge both on the fabrication technique and hydraulic resistance tolerances of the developed modules) was taken into account for each of the different discrete passive elements developed by Bhargava *et al.* (Bhargava et al., 2015).

In this sense, it is also relevant to highlight the importance of applying mathematical modeling and fluid dynamic simulation to the first stage of development of individual fluidic parts (Wu and Gu, 2011b). Such tools, that can integrate hydraulic characteristic estimation as described above or more complex fluidic descriptions, can help boost the development progress of microfluidic platforms (Ungerböck et al., 2013). This can be achieved, namely, by aiding in geometry optimization, evaluation of transport phenomena, determination and prediction of reaction (kinetic) parameters and mechanisms, and in analyzing experimental data (Ungerböck et al., 2013). Modelling provides a more targeted and therefore often more efficient strategy of device development and sensor design, which can result in faster, less wasteful, and more economical device development processes (Rosinha Grundtvig et al., 2017), (Gärtner, 2017). It also provides information for evaluation and choice of materials (either by modeling interaction between materials, absorption of molecules on the surface or elucidating the influence of properties such as, for example, thermal coefficients). The development of numerical simulation tools with a simple user interface might also contribute to spreading the use of microfluidics to other fields (e.g. environmental sampling, resource recovery, structural analysis of soils or buildings, etc.), including transferring their use to non-microfabrication specialists (Wu and Gu, 2011b), such as also proposed for 3D printing technologies. As suggested by Chiu *et al.* (2017), the assembly of the building blocks of a modular microfluidic system could be guided by software, taking into consideration both the requirements of the specific application as well as the characteristics of the building blocks available (Chiu et al., 2017). Bramsiepe *et al.* (2014) have suggested a process planning tool based on a systematic computer-aided and user-guided 5-step approach to evaluate the feasibility of microreactors for a given chemical process (Bramsiepe et al., 2014), which could also be applied to module selection in biotechnology by adopting different performance indicators that are relevant for biotechnological applications.

In Figure 9, two approaches towards widening the access to modular microfluidics are presented. As explained in this section of the paper, these two views are not only based on the availability of modular microfluidic unit operations but also on their standardization in terms of connectivity. This could significantly speed up the development of new diagnostic systems, the set up and fast optimization of a biocatalytic or synthesis multi-step reaction or the use in real applications of new sensors, by providing already proven and validated microfluidic system components. Furthermore, the use of standardized modules would facilitate the comparison of results attained between different groups since the same or similar modular platforms could be used to perform the assay. Moreover, in sharing optimized designs with the scientific community a faster advancement of the field itself could be obtained.

However, as also discussed in this section, a modular approach has some challenges which need to be tackled before either of the two approaches presented in Figure 9 can be attained. It is also important to highlight that modular microfluidics may not provide the best platform for a final device in some applications, where a higher degree of accuracy is required (e.g. with respect to analytics) or when contamination issues are a concern (e.g. medical devices, biological sample handling, diagnostics). Even though we assume that the user of such platform would be highly skilled, whether in academia or industry, we believe that the access to such systems (in the form of Microfluidic Modular Toolboxes) for the general public would contribute to a better awareness of microfluidics and its potential contributions to society, and thus higher acceptance of its use in diagnostics, biomedicine, food safety, etc.

2.3 Other considerations

Polymeric materials seem to provide the necessary characteristics, such as flexibility (both in terms of design and fabrication, as well as the final application) to benchmark microfluidics as the wide-range tool it was always intended to be.

One of the attractive features of polymeric materials for diagnostic platforms used in biomedical and clinical applications is their disposability due to their low fabrication cost (Wu and Gu, 2011b). These attributes decrease the risk of user contamination when handling potentially dangerous samples or of substances being analysed erroneously due to sample carry-over. However, for most applications in other fields, where contamination issues can be easily solved or are less critical, the use of disposable devices will lead to the generation of unnecessary and possibly difficult to handle waste (most materials used in microfluidic platforms and microsensors are not biodegradable (Luecha et al., 2011)). Furthermore, most microfluidic platforms consist of a variety of materials (including e.g. heavy metals and other compounds potentially toxic to the environment) assembled in an irreversible way, or at least in such a way that the different fractions are difficult to separate, thus increasing the difficulty in disposing of such devices in an environmentally sustainable way. The academic community should probably seek to develop multiple use platforms in such situations, as well as invest in biodegradable or transformable/reusable materials, especially when many microfluidic platforms may be used (e.g. screening of enzymes or process parameters). Zein (a prolamin protein from corn), poly (lactic acid) (PLA) (Mills et al., 2006), silk fibroin and gelatin are examples of biodegradable materials that can be used to fabricate microfluidic devices (Luecha et al., 2011). Zein has great potential as plastic substitute (Luecha et al., 2011), (Corradini et al., 2014), (Kokini et al., 2015), (Gezer et al., 2016) since it is biodegradable and can be produced from excess in the corn industry, adding value to a traditional bioethanol production process and also reducing waste in industry (Lawton, 2002). Zein is typically used mixed with other components (such as polyethylene, starch, antimicrobial agents, glutaraldehyde, formaldehyde, aliphatic alcohols), whose influence in terms of toxicity and biodegradability has not yet been studied extensively (Corradini et al., 2014). PMMA is another promising material to obtain “green microchips” since it can be reused after decomposition to methyl methacrylate (MMA) at high temperatures (Chen et al., 2008).

To conclude, besides the effort to use alternative, more environmentally friendly and/or reusable materials, the microfluidic community should strive towards building devices that can be disassembled easily. This would facilitate the reuse or disposal of the different parts of

microdevices, and thereby decrease the possible environmental impact that an intense use of this technology might bring.

Table 2 –Materials used for microfluidic platforms and their main characteristics (Mark et al., 2010),(Wu and Gu, 2011a),(Nge et al., 2013),(Ilescu et al., 2012),(Li et al., 2012),(Kangning Ren, Jianhua Zhou, 2013),(Gärtner et al., 2007),(Martinez et al., 2010),(Liana et al., 2012),(Yetisen et al., 2013),(Xia et al., 2016).

3. Towards a guide to development of standalone platforms

In order to develop a standalone multi-unit operations microfluidic platform, compatible with a wide range of applications, several considerations should be made. To illustrate the complexity of such endeavor, a guide for the development of a single unit operation microfluidic system is presented here.

In this work, the chosen unit operation is sensing, focusing on biomolecules and/or biological components, due to its relevance and variety of detection techniques. If microfluidic platforms are to be used widely, the integration of sensors and their validation as quantitative detection systems is of major importance. There are three main detection methods used in microfluidics: optical methods, electrochemical methods and mass spectrometry methods, of which optical and electrochemical methods are the most applied due to their selectivity and sensitivity. Other methods involve techniques such as nuclear magnetic resonance (NMR) spectroscopy and mechanical detection (e.g. quartz crystal microbalance (QCM) sensors or microcantilevers) (Wu and Gu, 2011b). Within each detection method there are several techniques, whose usefulness or applicability is highly dependent on the desired function of the device and where it will be integrated. Table 2 presents a summary of the main characteristics to consider when selecting a detection method for integration in a microfluidic platform.

Table 3 – Summary of the leading detection systems available for microfluidic applications. Integration capability is here assumed as the ease of miniaturization of the sensing system itself in order to be integrated inside any or most microfluidic structures. Portability relates to the miniaturization of required auxiliary equipment to perform the measurement (e.g. potentiometer, microscope, etc.).

Recently, even though new sensing technologies are discovered every year, there has been a shift in sensing research towards more efficient and hybrid integration of the sensing approaches by further developing already existing and validated sensors (Duval and Lechuga, 2013). The combination of different sensing technologies on the same device will widen its application, by increasing the number of targets it can monitor and/or quantify simultaneously.

The scheme in Figure 10 illustrates the major steps to be considered during the development of a new system for sensor integration. This scheme is divided in 3 steps:

- **Step I - development of the system's concept:** It is addressing a current need (of a potential client, a clinically relevant analytical device, isolation of an unstable compound or a research project) and involves the preliminary design, literature research for current technology, and preliminary concept tests in the laboratory. The concept of the device

should be concurrent with existing regulations in the field of application, especially when food or health related applications are planned (e.g. highly regulated by the Food and Drug Administration [FDA] and European Medicines Agency [EMA]). Further regulatory issues will not be considered here due to the complexity and variety of the subject. It should also take into consideration the end-users and their requirements for the device, as mentioned in more detail in (Bridgelal Ram et al., 2008) and (Shah et al., 2009). The more general the “need” identified, the more challenging the design of the system, but a wider use microfluidic chip might be achieved. In Figure 10 we use two specific, but hypothetical, examples (monitoring of blood glucose levels in real-time and inline monitoring of glucose concentration in a reactor), but a more generic need could be the development of a system that is adaptable for the on-line monitoring of several components;

- Step II – Sensor choice and fabrication: It includes an iterative choice and test of the sensor approach (and design) that is best matching the need defined in Step I, as well as the selection of chip material and fabrication method. These choices are often limited by the available or accessible technology and materials, as well as their cost of operation and use.
- Step III – Sensor integration: It involves the assembly of the final system for integration of the chosen sensors, based on the desired final application and type of device operation. The type of integration strategy should be defined from the concept step (step I), since it can limit the used materials or fabrication methods. At this point, further improvement or alterations of the components (developed during step II) of the prototype might be required. Since the final goal is the commercialization or wide use of the device, scaling of fabrication towards mass production should also be considered during development of the prototype.

In this work, we intend to provide a simple and useful guide to sensor integration in microfluidic systems. Furthermore, we would like to highlight the importance of taking a holistic approach to device design, as well as the relevance of thinking about connectivity to other systems during the design phase. When developing a multi-unit operation platform or a system compatible with other multi-unit operation platforms, these steps should be followed first for each unit operation, and then for their consecutive integration with each other, until the whole-platform integration is achieved. All the time, one should keep all the considerations presented in Figure 10 (e.g. final user, type of sample, location, etc.) in mind.

Figure 10 – Decision analysis cycle scheme for developing microfluidic systems for sensor integration, and its application to two hypothetical case studies: (i) a hypothetical portable glucose electrochemical sensing device and (ii) a hypothetical inline glucose monitoring device.

3.1 Application of the decision analysis cycle scheme: forward application to two hypothetical case-studies and retrospect application to three literature case-studies

The developed decision analysis cycle scheme (see Figure 10) proceeds from the identified need, market niche or target application, across the different steps of prototype

development until a satisfactory device is obtained. It is focused on sensor integration, since it is a common challenge within microfluidic applications, but it could also be applied to the development of other types of systems. Taking as example a few of the unit operations provided in Table 1, if a mixing or fluid handling unit is being developed, the first choice to make in Step II would be the type of mixing (e.g. magnetic) or type of pump (e.g. electroosmotic) that is more appropriate towards fulfilling the identified need (in Step I). The type of mixing or pump chosen would in turn constrain the materials and fabrication techniques that can potentially be applied. On the other hand, if the intention is the concentration or purification of a target compound, then material (due to compatibility to the solvent or functionalization to the biological element) and channel design (to achieve the concentration or purification) are the first priorities in Step II. Fabrication can be highly relevant in sorting applications when channel topography and/or hydrodynamic focusing are used. Thus, even though the order of the different elements referred to in Step II may vary depending on the need when sensing is not the targeted unit operation, these elements are still among the first considerations to be made.

Step III involves the holistic analysis of the choices made in the previous steps but also the application of the device developed in a real setting. Step III involves the holistic analysis of the choices made in the previous steps but also its application in a real setting. Failure at this step will result in the alteration of one or several of the choices previously made, be it the type of sensor, the channel design, the type of connectors, the assembly strategy, the chosen mode of use or the fabrication strategy. Failure due to insufficient detection ability or a too high detection limit will imply an optimization of the sensor used (if possible), the use of different sensing layers or sensor geometry or the application of an entirely different sensing strategy, which can result in a re-design of the device. Failure due to leakage, velocity lower than desired or poor mixing will result in an improvement of the channel assembly, connector design or size, better or different pump, or different channel design. By defining proper goals for device performance, its characteristics can be iteratively improved through a step-wise analysis and optimization of its components, as presented in the feedback loop in Figure 10.

However, the re-definition of the microfluidic platform may even require a re-analysis or re-consideration of the final use or type of sample defined in Step I. For example, an added sample dilution may be considered necessary to facilitate flow or quantification of the target compound thus adding one more unit operation to the platform. Another example involves the choice of an altogether different sample to facilitate quantification of the target compound. For example, instead of saliva, particles in the breath may be considered more appropriate. The latter would then imply a complete re-design of the platform, even if material, sensor and fabrication strategy are kept. This indicates, as mentioned previously, that Step I, where the system's concept is defined, is often the most complex and crucial of all the steps.

3.1.1 Forward application to two hypothetical case-studies

The application of the provided guide is performed for two hypothetical case studies: (i) a portable glucose electrochemical sensing device; and, (ii) an inline glucose monitoring device. For each case study, the conclusion of the considerations presented at each step is shown in Figure 10.

For case study (i), in Step I, the key concept to address is the need of monitoring glucose in blood. This is achieved by defining a device capable of performing measurements in real-time that should be portable and able to draw samples subcutaneously. This concept is achieved by considering the patient as the end user, the importance of constant monitoring of glucose levels in diabetic patients, and therewith of portability, and the best sample format as blood. In Step II, the development of such a device begins by choosing the best sensing approach, and the appropriate materials and fabrication methods. In the chosen example, an electrochemical sensor is selected due to its ease of miniaturization and the extensive available knowledge on applications of electrochemical sensors for glucose monitoring. Then, considering that the device will be in close and continuous contact with the patient's skin, a biocompatible material was chosen (PDMS). Furthermore, when using some biomolecules, such as the enzyme glucose oxidase (if certain mediators are used), oxygen is required for the reaction and can also be used as a target analyte. Therefore, the use of PDMS is further highlighted due to its permeability to oxygen. The choice of material and the dimensions of the device (also defined in Step I) would then limit the choice of fabrication methods, together with the available methods for the device developer. Finally, in Step III, the combination of the different already described parts (through the use of a casing, for example, for easier re-use or substitution of sensors or channels) and the test of the prototype occur. This will provide a validation on whether further development or alteration of one or more of the described parts is required, before the final device is obtained.

For case study (ii), the key notion guiding the concept development is the continuous monitoring of glucose in an outlet stream of a lab scale fermenter. Thus, the device needs to be robust, withstand relatively high pressures/flowrates, and be easily used by an operator. Furthermore, since the samples contain a complex matrix (media) with organisms, the system needs to be able to withstand sterilization and be connected to a sample pre-treatment device, where biomass is removed to minimize fouling of the sensors. In Step II, an electrochemical sensor is selected due to its price (in case substitution is required due to fouling) and straightforward connectivity and monitoring with electrical interfaces. Then, due to the required robustness of the device and compatibility with cleaning-in-place procedures, stainless steel is chosen as the platform's material. This limits the available fabrication techniques, of which milling offers a relatively lower cost and device completion time. In Step III, the combination of the different components is achieved, with the test on the device's robustness, the influence of biofouling on sensor performance, as well as the capability of sample pre-treatment to reduce this effect. Validation of device performance is achieved and the need for alterations assessed, as described in Figure 10.

3.1.2 Retrospect application to three literature case-studies

To further demonstrate the universality of the developed decision analysis cycle scheme, three devices developed by other research groups were interpreted based on the published results following the guide in Figure 10. The chosen devices below are at different stages of development and from diverse scientific fields.

Example 1: Babikian *et al.* (2015) developed a wearable device capable of performing isotachopheresis (ITP), which encompasses an electrochemical assay capable of isolating and purifying small biomolecules in low abundance from biological samples (Figure 11) (Babikian

et al., 2015). The objective was to develop an integrated bioflexible electronic device (IBED) that can be worn on the body, to address the current need for “low cost and high-quality health care that can be provided at the point of care”. Thus, the device should: (i) allow access to communication networks (e.g. wireless, Bluetooth, internet) to “provide health care to remote or poor areas”; (ii) be compatible with large-scale production techniques to enable a low cost per device; (iii) integrate several components capable of performing the target bioanalysis; and, (iv) be biocompatible, flexible and thin so it can be worn on the skin. Therefore, the characteristics required for the design of the device in Step I were thus defined. Within Step II, the materials and fabrication techniques that best fitted the requirements established in Step I were chosen. In this case, Babikian *et al.* selected two polymers as the main components of the device: polyester, as the device support material, and PDMS, as the biocompatible material that forms the microchannel in contact with the biological sample. Both polymers can be coupled even if fabricated with different techniques, and can be made with a small thickness. To achieve the target analysis, both electronic and optical components were required, so aluminum electric trace, which is robust when subjected to bending, was chosen as the material for the contacts. Two blue LEDs and electrodes were also integrated as excitation elements and electric field generators. In Step III, the overall performance of the device was adequate, but the need for a different electrode material, more resistant to electrolysis effects (e.g. platinum instead of aluminum) was identified. Also, in the next iteration of the development of the device the authors would like to implement detection on the chip by integration of optical detectors and optimizing the on-chip optics of the device.

Figure 11 - Bioflexible electronic device developed by Babikian *et al.* (2015) (Babikian et al., 2015). The figure presents the device with integrated electronics and microfluidics (A) worn on the body (B).

Example 2: Moon *et al.* (2007) developed a disposable somatic cell counter for quality assessment in raw milk (Figure 12) (Moon et al., 2007). The reference method for determining somatic cell count (SCC) is direct microscopic analysis, which requires sampling, sample transport to a laboratory facility and then analysis by trained personnel, increasing the time between sampling and result availability, as well as errors associated with the operator. Thus, a standalone, disposable and automatic system that enabled operator independent analysis was highly desirable. In order to achieve this, in Step II, PMMA, a biocompatible polymeric material, was chosen for the microfluidic chip, which was designed and modified with gas plasma in order to allow capillarity as the sample loading method, to facilitate fluid handling during analysis. As sensing element, a CCD camera coupled to an image analysis program, a laser and a light source were used. Using an optical detection close to the reference method facilitates validation and comparison with the standard method as well as acceptance by the end-users. By using a white light and a laser, both cell morphology and the stain for cell DNA were observed, so a more comprehensive analysis of the sample was achieved. In Step III, the optical components and a personal computer (PC) were integrated in an easily transportable casing with integrated LCD screen for easy operation of the device. The final measurement apparatus presented a reproducible performance, with accuracy within the range for the standard method and similar performance to other conventional automatic instruments. A next iteration of such device could include a miniaturization of the optical components of the

device towards a truly portable piece of equipment that could enable on-site counting of somatic cells.

Figure 12 – Disposable somatic cell counter for quality assessment in raw milk developed by Moon *et al.* (2007) (Moon *et al.*, 2007). The figure presents the portable C-reader (A) and two disposable microfluidic chips, one for loading of a single sample (with one channel) (B) and another for 2 sample loading (with two channels) (C). Image reprinted and modified from J. Dairy Sci. 90, J.S. Moon, H.C. Koo, Y.S. Joo, S.H. Jeon, D.S. Hur, C.I. Chung, H.S. Jo, Y.H. Park, “Application of a New Portable Microscopic Somatic Cell Counter with Disposable Plastic Chip for Milk Analysis”, p. 2253–2259, copyright 2007 with permission from Elsevier.

Example 3: Zou *et al.* (2016) developed a microfluidic device capable of monitoring the ethanol concentration in fermentation processes (Figure 13) (Zou *et al.*, 2016). Ethanol above certain concentrations is harmful for microorganisms (e.g. inactivating the yeast used for ethanol production at values above 10% (v/v)), so its control within a certain range is essential for efficient production. Standard methods for ethanol quantification are based on bulky equipment, with long offline analysis times and it requires specialized personnel. Therefore, in Step I, the authors identified as a target the development of a fast, on-line, low-cost and simple-to-use device. Furthermore, since already existing analytical devices (e.g. biosensors based on enzymatic reactions) are limited to certain temperature and pH ranges, small ethanol concentrations or shorter operation times, Zou *et al.* also intended to develop a device capable of operating in a wide range of process conditions. To achieve this, in Step II, they selected a functional membrane (made of poly(N-isopropylacrylamide) nanogels in polyethersulfone), which is responsive to ethanol, as the sensing element. This membrane presents a different permeability at different ethanol concentrations, increasing the flux of solution through the membrane with increasing ethanol concentrations. This flux change is then used as a visual detection of the ethanol concentration. Since the membrane requires constant immersion in aqueous solutions during storage and to facilitate monitoring of fermentation broth, the authors encapsulated the membrane in a microfluidic channel made of PDMS. In Step III, the assembled device was tested with solutions with known concentrations of ethanol, increasing concentrations of ethanol and ethanol production fermentation broths. In the latter, the performance was very similar to the one of a gas chromatograph (GC). Since PDMS is highly flexible, bending of the assembled device might lead to tearing of the functional membrane, so in a next iteration it might be interesting to place the device in a rigid casing or build the microfluidics platform from a more robust material (e.g. polycarbonate, or even steel). Using another material for the microfluidics would also eliminate concerns with regards to the PDMS permeability to ethanol, which is known to occur. Furthermore, the membrane performance is temperature dependent, which might not be a problem when monitoring a fermentation process due to the similarity between the optimal membrane temperature and fermentation temperature. But this could be further improved, as the authors also mentioned, by modifying the membrane with hydrophilic or hydrophobic co-polymers. Also, detection of flux is performed visually, which might lead to inaccuracies, so coupling this device with a flowmeter might increase its performance.

Figure 13 – Setup for monitoring ethanol concentration developed by Zou *et al.* (2016) (Zou *et al.*, 2016). The figure presents the circulation loop flow diagram (a), a photograph of the actual setup (b) and a magnified photograph of the microfluidic membrane device (c). Image reproduced from Zou *et al.* (2016) (Zou *et al.*, 2016) with permission of the Royal Society of Chemistry.

3.2 Further considerations

The discussed case studies allow a clear presentation of the suggested guidelines. However, they are a simplification of the real applications of the described devices.

In reality, the extremely strict regulations involving health applications would further limit the types of structures, materials and fabrication methods that can be applied in the case of case-study (i). A close collaboration between microfluidic developers, physicians and health regulation agencies could be established (such as ISO 13485:2016 for Medical Devices) in order to satisfy demands related to both health & safety and the patients' quality of life.

PDMS as a biocompatible, optically transparent, gas permeable and easy to mould material has been for many years the perfect low-cost prototyping medium at the academic level (Whitesides, 2006), (Johnston et al., 2014). It allows a great flexibility of channel and unit operation design and can bond with glass or silicon surfaces under mild conditions (thus not affecting deposited sensors performance) (McDonald et al., 2000). Its surface can also be functionalized easily (Zhou et al., 2012), further increasing applicability in testing new ideas, sample processing techniques or detection methods, especially in bio-oriented research. It was thus chosen as a well-known example of a biocompatible polymer material, already used in some commercial devices (e.g. I-Stat and Fluidigm's Topaz chip (Carlborg et al., 2011)) to illustrate the guide's application in case-study (i). However, its low fabrication reproducibility, reduced hardness (cannot sustain high pressure conditions and is susceptible to structure deformation (Johnston et al., 2014)) and poor compatibility with organic solvents (swelling in the presence of e.g. ethanol and isopropanol (Whitesides, 2006)) render it less desirable for commercial applications that need to be highly robust and compatible with mass production methods. Another issue with PDMS is the dissolution of small organic analytes in the bulk PDMS, which can lead to sample loss and modification of PDMS with time (McDonald et al., 2000). For commercial applications, thermoplastics, such as Poly(methyl methacrylate) (PMMA), Polycarbonate (PC), Polystyrene (PS) and Cyclic Olefin Copolymer (COC), are usually used since they are compatible with high-throughput prototyping techniques such as injection moulding and hot embossing (Sollier et al., 2011), which in turn enable to greatly decrease the cost of microfluidic devices and thus increase their commercialization (Gärtner, 2017). Polystyrene is especially relevant in cell biology, since it has been used for a long time already at laboratory scale (Sackmann et al., 2014). However, their fabrication techniques are usually more expensive and slower than polymer casting (usually applied to PDMS) and bonding strategies to other materials are also not as varied. New non-lithographic mould fabrication approaches to PDMS (e.g. Print and Peel (PAP), xurography, direct laser plotting) have also been developed which can increase its fabrication compatibility with mass production, but can only achieve micrometer-sized structures (Faustino et al., 2016). Alternatives to PDMS as a prototype material that are also applicable in academia include Thermoset Polyester (TPE), Polyurethane Methacrylate (PUMA) and Norland Adhesive 81 (NOA81) (Sollier et al., 2011). These materials require a two-step cure, the first involving UV exposure, but result in highly reproducible structures with optical transparency and better solvent compatibility than PDMS. They also enable high bonding strengths to a wider variety of materials, such as dielectric and metallic mirrors (Sollier et al., 2011). TPE, PUMA and NOA81 also become hard after curing thus being applicable under high pressure conditions and more compatible with commercial high-throughput techniques. However, they cannot be

applied to pneumatic valves and moving parts due to lack of elasticity. In terms of biocompatibility, PUMA and NOA81 exhibit higher cell viability than PDMS (Sollier et al., 2011). Off-stoichiometry thiol-enes (OSTEs) present characteristics in between PDMS and commercial thermoplastics, allowing for higher control of surface modifications and tuning of mechanical properties of the final structure in a scalable fabrication process (Carlborg et al., 2011). OSTEs thiol-ene ratios can also be tuned to allow fabrication of flexible devices and thus produce mechanically actuated components (Carlborg et al., 2011). Other materials, intended as a bridge between PDMS and thermoplastics in terms of high-throughput fabrication and material characteristics, are styrene-ethylene-butylene-styrene (SEBS) block copolymers (Domansky et al., 2017). For biomedical applications, the focus has shifted from PDMS-like materials towards bio-based materials such as silk and hydrogels, due to their biodegradability and surface and topographical properties (Konwarh et al., 2016), (Zhao et al., 2016). These materials, however, require different fabrication approaches, which are not easily scalable (Konwarh et al., 2016), (Zhao et al., 2016).

Regarding case-study (ii), the integration of such a device might be easy to implement into a laboratory scale reactor, as presented, but the final goal would always be its use in pilot or industrial scale reactors. For such larger scale reactors, once again, tighter regulations exist, mainly related with the existing inlets for the reactors and the costs associated with the potentially necessary modifications. These more rigid regulations would very likely add more iteration steps in the development of the platforms.

The presented examples of published microfluidic devices enabled a more concrete implementation of the different steps proposed in the developed guide (see Figure 10). However, not all the reasoning guiding the selection of the used components or materials is presented in the articles. These design choices are often ruled by the available options in the research facility, especially in proof-of-principle systems, rather than by the end application, as mentioned in the stepwise guide. Moreover, since the next iterative steps in device development were not always clearly presented in the articles consulted for this manuscript, we suggested some alternatives that seemed feasible, but might not be the most appropriate for the field of application in question.

Furthermore, simply fabricating devices that can be connected more easily might not be enough to increase their use in the market. As previously mentioned, device validation with currently used analytical methods, which can vary greatly across fields, is of utmost importance to gain trust from the stakeholders (the final customers and/or investors). A broadly applicable or multi-use device would have to be validated by all analytical methods commonly used in each specific field, which due to its characteristics, namely dimensions, might be challenging to achieve, and should therefore also be considered as a main objective from Step I. Once validated, such device could in turn become a reference analytical tool of the field, with a simpler application and a lower price. Microfluidics can become an interesting analytical tool, more accessible to the general public, both in terms of cost, portability, and space used, but also in terms of simplicity of use.

Additionally, it is relevant to highlight the importance of the end-user in the development of microfluidic platforms. When the final user of the platform is a patient or

someone with little or no training in the technology or field in question, the final device needs to be not only easy to assemble, but “fail-proof”. This means it should have a robust operation in order to withstand possible operation errors, such as wrong types of samples, labels or reagent concentrations, and present a higher number of redundancies and security protocols, being at a high level of developmental maturity. Such a device should provide limited options in terms of operation and minimize required external input (so to minimize errors from the operator), and provide sufficient and detailed protocols and operation guides. Moreover, if samples from the patient are needed (e.g. in the case of diagnostic devices), the sampling procedure should preferably be non-invasive or at least reduce invasive sampling as much as possible, and avoid sample cross-contamination (if measurements of multiple samples are required for monitoring of a disease status for example). Such a platform should also provide already treated data, and if possible guidelines of steps to proceed or suggestions of what the data might signify (Martin et al., 2000). Patients and non-specialized personnel would probably prefer a standalone platform relative to several modules that require assembly. On the other hand, if the end-user belongs to a research or medical laboratory, or industry, although the same requirements in terms of validation and safety are expected, the flexibility in terms of operation modes and assembly structures is higher. Also, additional analytical components and a lower or no data processing effort are expected.

We would also like to emphasize that, in this interdisciplinary field, the solution to a given “need” might be found in a seemingly un-related field or approach. Thus, collaboration and communication between disciplines is also crucial, if microfluidics is to become a more widely used technology (Sackmann et al., 2014).

Finally, even though this has not been discussed in detail here, it is also important that scale-up of the developed modular microfluidic platforms is considered during development. As mentioned, modular-based platforms offered as a toolbox can provide an excellent basis for optimization studies, but the transfer of the knowledge obtained with such platforms to a larger scale is not linear. In this sense, and in the case of small-scale productions (e.g. production of certain APIs) it may be easier to scale-up the developed platform, than to transfer the gathered properties into batch, be it a pilot or production scale reactor. Studies such as the one presented in (Kockmann et al., 2011) for a single channel microreactor provide a good direction on reaction and fluidic characteristics that are relevant to consider when scaling and also explain the effect of scale on reactor performance, since scaling-out may not always be the best approach in terms of platform robustness. In Bieringer *et al.* (2016) (Bieringer et al., 2016), an overview of the relevant characteristics and corresponding measurable parameters during scale-up of unit operations is also discussed and presented.

4 Future perspectives

Modular approaches have the potential to revolutionize how research and industrial production are performed, as demonstrated by the increasing investment in module manufacturing projects (e.g. AIChE Rapid Advancement in Process Intensification Deployment (RAPID) Institute in US, F3 Factory project in Europe). Furthermore, several companies, such as Novartis, have begun switching from batch to continuous production, or begun providing

equipment to enable that switch, such as GEA and Siemens (Gürsel et al., 2017). This institutional change towards continuous processes will result in an increasing use and demand for microfluidic-based reactors, separators, mixers and entire fluidic networks. In the biotechnology field, the technology is not yet ready for application in small-scale plants, but some European projects have developed interesting devices for application at the screening phase in the laboratory (e.g. BIOINTENSE - Mastering bioprocess Integration and intensification across scales, EUROMBR - Application of microbioreactors (MBR) in bioprocess development, MICROBUILDER - An integrated modular service for microfluidics). The potential for microfluidic and microstructured based modules is thus becoming noticeable and is expected to grow considerably in the next 5 years.

The use of modular-based microfluidic platforms would greatly benefit from the establishment of design databases and wide agreement within the community of using similar or compatible connector approaches. An increase in the communication between individual groups would also enable a better compatibility in terms of operating flows and conditions, as well as to accelerate the testing of a given device with diverse samples, points of use, operating conditions or final users. This would in turn thus decrease the time to market of useful and necessary devices. This seems to be the current direction in flow chemistry, where there have been several efforts in terms of developing modular-based reactors and miniaturized downstream unit operations towards the small-scale production of fine chemicals and pharmaceutical compounds (Buchholz, 2010), (Löb, 2013), (Bramsiepe et al., 2014), (Adamo et al., 2016), (Mardani et al., 2017). The further development of modular-based process planning software to include a database of the previously mentioned modular unit operations, is already under way in the chemical industry (Bramsiepe et al., 2014). This will further contribute to the acceptance of microfluidic modules for laboratory studies and small-scale production and in turn to its marketability as a process optimization and production tool. The change to modular-based production enables an easy switch to continuous manufacture, which is one of the current challenges in the chemical and biotechnological industries. In this sense, the Food and Drug Administration (FDA) has recently approved continuous manufacturing of pharmaceutical compounds, such as the HIV drug Prezista (Gürsel et al., 2017).

The further miniaturization of electronic components (especially, power sources, data acquisition systems, but also analytics such as Raman spectroscopy and NMR) and fluidic components will also contribute significantly to an easier commercialization and acceptance of microfluidic devices, with a significant impact on field analysis. Additive manufacturing, as previously discussed, will also transform the approach to microfluidic device development by enabling new 3D structures, and a more immediate connection between fluidic and mathematical simulations. As 3D manufacturing equipment becomes more affordable and its use more widespread, the existence of open-access module design databases will become more relevant. This will in turn further promote collaborative development of modular-based platforms, since they can be easily reproduced in distant laboratories, institutes, companies and eventually households.

Fluidic and mathematical modeling will also play a bigger role in the development and application of modular platforms, which will be facilitated through the access to online databases on device design and available experimental measurements.

Certain fields, such as genomics, proteomics, single cell manipulation and analysis, microphysiological system on a chip (e.g. “organ-on-a-chip”), rare species detection and diagnostics, flow chemistry, for which microfluidics provides the perfect tool due to its inherent characteristics, will continue to grow in terms of use of microfluidic platforms in fundamental research, as stated by (Chiu et al., 2017). We envision that, as previously occurred for DNA sequencing, and recently for flow chemistry (small production plants), more and more of the microfluidic-based systems used in the laboratory will be transformed into commercially available platforms. This will encompass, as previously discussed, the development of simple to operate platforms, whose application to a given research project (e.g. drug discovery and drug clinical trials) or process (e.g. in situ production of personalized medicine) surpasses other technologies in terms of attainable outcome. For these areas, the small volumes and controlled fluid velocities associated with mass transport dominated by diffusion translate into highly defined sample volumes, reaction times, residence time and gradients. As such, the technology is extremely useful for analytical quantification, clinical chemistry and bio-assays (e.g. cell or antibody-based assays), especially in situations where analytes are highly diluted or only available in a small sample volume (Chiu et al., 2017). The small dimensions and gradient generation enable accurate control and study of the environment of single or small populations of cells (Zare and Kim, 2010). The possibility of defining compartments with controlled environments in even smaller volumes such as droplets and their rapid sorting and analysis allows for a fast study of mutagenic variants, or to perform a high number of genetic mutations to achieve, for example, better biocatalysts (Kintses et al., 2012). Also, as mentioned already, the high degree of fluidic control and fast heat transfer, enables producing compounds that are otherwise extremely hard or dangerous to obtain, as well as to operate at well-controlled conditions (Hessel et al., 2013).

For other areas, such as for example, environmental monitoring, where samples can contain a large variety of particles and biocompounds, as well as present a diverse range of viscosities, new technologies or strategies for sample preparation must be developed. The development of standalone modular microfluidics may be a way of dealing with the diversity of samples such platforms need to be able to handle. For example, a modular microfluidic field toolbox could include unit operations specifically developed to deal with higher viscosity or solid samples. To guarantee reliable results, such a field investigation toolbox could also entail a rigid casing inside which the different modules could be assembled and protected from harsher environmental conditions and thus increase robustness of the final chosen module assembly. We believe that the current need for more field monitoring approaches coupled with the potential portability of microfluidic platforms will drive such development in the next 10 years.

5 Conclusion

In this work, we propose a step-wise guide to develop standalone microfluidic platforms. Its application to two case studies focusing on sensor integration, as well as the analysis of three published microfluidic systems, are presented, as examples of the usefulness of the method. The guide highlights the main considerations to take into account when conceptualizing such a platform, as well as the precautions to take during fabrication.

Limitations regarding country or field regulations, as well as in terms of materials and methods available to the researcher are also considered. Furthermore, a short review of the various materials used in microfluidic platforms and the main fabrication techniques for each material are also included. This review was introduced in order to offer further recommendations when performing some of the described choices in the presented decision analysis cycle scheme in Figure 10.

Moreover, we describe some of the characteristics that have hampered the growth of microfluidic applications in the market, especially in terms of translation into final products, such as the complexity of operation, the low adoption by non-microfluidic specialists and the current commercial development and business models. Additionally, we offer and discuss potential solutions to one of the main identified challenges, low platform flexibility, such as the diversification of the purpose of each platform, and increase of the connectivity of microfluidic components to other systems through standard connections. In this regard, we propose two models for commercialization of modular microfluidic systems intended to cause their more widespread acceptance by non-specialists and increase their use and flexibility in academia. We also suggest that the key solution to some of the challenges identified in microfluidics can be resolved by applying techniques inspired by other fields or solutions found for different types of problems.

All in all, with this review, we intend to provide a brief overview of microfluidics as a tool to promote technological advances (enabling technology) and the possible impact on society, by highlighting some of the main challenges the adoption of microfluidics technology faces and its current place in the market. Moreover, we present a simple, clear and detailed approach towards developing microfluidic platforms that we believe may be useful for increasing the future impact of microfluidics on society. The proposed approach can also serve as a starter guide to researchers that are new to the field.

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Table 1 – Summary of the presented unit operations currently available for microfluidic chips.

Unit operation	Function	Examples
Fluid handling	Flow definition and control	<ul style="list-style-type: none"> • Miniaturized and integrated pumps (Sabourin et al., 2013); • Magnetically actuated stirrer-based micropump (Kimura et al., 2015); • Electroosmotic-based pump (Gao and Gui, 2016); • Screw-actuated valves (Zheng et al., 2009); • Braille display actuated valves (Gu et al., 2007); • Capillary-based valves (Madadi et al., 2015), (Boyd-Moss et al., 2016); • Hydrogel-based valves (Wang et al., 2005), (Ter Schiphorst et al., 2015), (Boyd-Moss et al., 2016);
Mixing	Combination or blending of two or more substances or compounds	<ul style="list-style-type: none"> • Passive mixing, such as channel topology (Liau et al., 2005), contact area increase (Lee et al., 2011), lamination (Gürsel et al., 2017), coiled flow inverter (Klutze et al., 2015) or stimuli-responsive hydrogels (Prettyman and Eddington, 2011); • Active mixing, such as acoustically-induced microstreams, dielectrophoretic micromixers, electrokinetic actuation (Gao and Gui, 2016), velocity pulsing and magneto-hydrodynamic flow have also been thoroughly developed and applied (Lee et al., 2011)
Dilutions	Definition of gradients of a target substance	<ul style="list-style-type: none"> • Droplet-based (Niu et al., 2011); • Peristaltic mixing (Rho et al., 2016);
Sample concentration	Increase the amount of a substance or quantity of a particle per volume of solvent or media	<ul style="list-style-type: none"> • Adhesion to the channel walls (Stott et al., 2010), (Jing et al., 2013); • Functionalized magnetic beads (Pereiro et al., 2017);
Filtration/purification	Removal of contaminants or separation of target compound/particle from solvent or media	<ul style="list-style-type: none"> • Size-based (Madadi et al., 2015), (Sarkar et al., 2016); • Channel functionalization (Stern et al., 2010); • Immunomagnetic separation (Chang et al., 2015); • Adhesion to silica (Shaw et al., 2013); • Solid-phase extraction (Park et al., 2015); • Liquid-liquid extraction (Gürsel et al., 2017);
Sorting	Isolation of target compound/particle	<ul style="list-style-type: none"> • Dielectrophoresis (Niu et al., 2007), (Adam R Abate et al., 2010), (Frenzel and Merten, 2017); • Electrostatic charging (Link et al., 2006); • Optical tweezers or traps (Wang et al., 2011); • Fluorescent activated cell sorting (FACS); • Membrane valves (Adam R. Abate et al., 2010); • Surface acoustic waves (Wang and Zhe, 2011); • Magnetophoresis or magnetic activated cell sorting (MACS); • Channel topography (Hsu et al., 2008); • Inertial or hydrodynamic focusing or affinity approaches (Z. T. F. Yu et al., 2014);
Sample amplification	Replication and increasing quantity of target molecule	<ul style="list-style-type: none"> • Micro PCR (Schaerli et al., 2009), (Shaw et al., 2013); • Micro MALBAC (Z. Yu et al., 2014); • Micro NASBA (Dimov et al., 2008);
Incubation	Extended residence time for reaction, labelling or cell growth	<ul style="list-style-type: none"> • Droplets in chambers (Theberge et al., 2012), (Mary et al., 2011); • Long channels (Adam R Abate et al., 2010);

Detection	Identification and quantification of the target compound	<ul style="list-style-type: none">• Dielectric (Niu et al., 2007);• Size changes (e.g. cells);• Capacitance changes (Bürgel et al., 2015);• Stroboscopic epifluorescence imaging (Hess et al., 2015);• SERS (Quang et al., 2008);• FT-IR microscopy (Polshin et al., 2014);• Organic photodetectors (Lefèvre et al., 2015);• Nano-wires (Stern et al., 2010);
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Table 2 –Materials used for microfluidic platforms and their main characteristics (Mark et al., 2010),(Wu and Gu, 2011a),(Nge et al., 2013),(Ilescu et al., 2012),(Li et al., 2012),(Kangning Ren, Jianhua Zhou, 2013),(Gärtner et al., 2007),(Martinez et al., 2010),(Liana et al., 2012),(Yetisen et al., 2013),(Xia et al., 2016).

Material	Inorganic	Polymer	Paper
	e.g. Silicon, glass, low-temperature co-fired ceramics (LTCC)	e.g. Polydimethylsiloxane (PDMS), Polyfluoropolyethers, Poly(methyl methacrylate) (PMMA), Polystyrene (PS), Cyclic-olefin copolymer (COC), SU-8	e.g. Whatman no. 1
Fabrication strategies	Batch	Batch or continuous	Batch
Fabrication techniques	Semiconductor industry techniques (etching, lithography, bonding, powder blasting and chemical or physical vapor deposition)	Hot embossing, injection molding, soft lithography, thermoforming, laser ablation, micromachining and photolithography	Inkjet and solid wax printing obtaining hydrophilic channels bound by hydrophobic barriers and either sealed with thin polymer sheets or left open
Smallest dimension	< 100 nm	< 1 μ m	~200 μ m
Material cost	High	Low	Low
Fabrication cost	High (during development) Low (during mass production)	Low (except for prototyping in the case of injection molding and thermoforming)	Low
Channel characteristics	Hydrophilic, charge stable, defined walls, limited 3D capability	Generally hydrophobic, channel definition dependent on polymer and fabrication strategy, moderate to high 3D capability	Amphiphilic, not very defined channels, moderate 3D capability
Surface functionalization	Yes	Yes	Yes
Integration	With electronic systems (e.g. for data acquisition) or electrodes (for detection)	With electrodes (by deposition onto polymer)	With electronic and magnetic systems, and electrodes (by containing electrical conducting inks)
Combination with other materials	Glass and polymers (transparent materials)	Glass, silicon, other polymers	Electrical conducting inks, carbon or metals (silver, gold, etc.)
Functional elements (e.g. valves and pumps)	Yes (complex fabrication)	Yes (simple to complex fabrication depending on technique)	Yes
Advantages	High chemical stability, known surface and insulating properties, high thermoconductivity, high aspect ratio channels	More resistant to mechanical shock, high to low oxygen permeability, easy bonding strategies, less stringent cleaning techniques, disposability, biocompatibility, transparency to most wavelengths	Highly porous matrix, fluid flow through wicking, low cost, easy reagent storage on channel (by dried spots), easy assembly of multiple operation units (e.g. sample metering, filtering and separation), disposability, visual readout
Limitations	High cost of development and fabrication, fragile, low oxygen permeability, requires annealing at high temperatures	Low to high resistance to organic solvents, water evaporation	Challenging precise liquid handling, sample retention during transport, relatively high limit of detection (LOD), limited detection techniques
Commercial availability	Yes	Yes (genetic and molecular biology analysis, protein crystallization, immunoassays)	Yes (qualitative output)

Table 3 – Summary of the leading detection systems available for microfluidic applications. Integration capability is here assumed as the ease of miniaturization of the sensing system itself in order to be integrated inside any or most microfluidic structures. Portability relates to the miniaturization of required auxiliary equipment to perform the measurement (e.g. potentiometer, microscope, etc.).

Detection method	Advantages	Challenges	Integration capability	Portability
Optical	Fast response; High sensitivity; Compact; Usually contactless; can allow for real-time monitoring and spatially resolved imaging;	Usually dependent on microscopy equipment; May require labelling;	High	Usually low, but can be high if CCD cameras or mobile phones are used
Electrochemical	Can allow for real-time monitoring; can be applied to most biological and chemical samples; potentially low costs in terms of fabrication;	Requires the presence or generation of an electroactive species; difficult miniaturization of measurement equipment; short shelf life of most biosensors; requires control of ionic concentrations pre-experiment;	High	Medium, if measurement systems are miniaturized
Mass Spectrometry	High sensitivity and selectivity; very low detection limits; can be label-free; requires low electrical operation power;	Long analysis time; bulky detection equipment; extensive sample preparation;	Low	Very low
Magnetic	Highly specific (reduced sources of magnetic behaviour in nature, for magneto-resistive sensors); allows for studying behaviour of atoms and molecules (in the case of NMR); No need for optical accessibility (in the case of NMR);	Requires labelling of the target samples and/or very strong magnets; requires expensive fabrication methods; limited reaction time scale (for NMR);	High	High, if measurement systems are miniaturized (Lee et al., 2008)
Mechanical	Usually label-free detection; sensor integration performed during fabrication (monolithic);	Sensitive to damping effects in the presence of liquid; long detection times; Complex fabrication	High	Medium to high, if measurement systems are miniaturized

Glossary

Glossary	
Microfluidics	“The science and technology of systems that process or manipulate small (10^{-9} to 10^{-18} litres) amounts of fluids, using channels with dimensions of tens to hundreds of micrometres.” (Whitesides, 2006). In this paper, we do not focus on paper microfluidics, which presents some specific characteristics which are out of the scope of this review, due to the difficulty of integrating paper-based microfluidics with other types of microfluidic systems.
Unit operation	Basic step in a process that entails a physical change, a chemical transformation or quantification/detection of one or more components involved in the process. The definition used here is different than the one usually applied in the chemical engineering field, where a unit operation only involves a physical change, while a unit process involves the chemical transformation. Using unit operation to address both unit operation and unit process as well as monitoring enables a simplification in the text of this manuscript when describing different microfluidic systems.
Microfluidic system, microsystem or chip	Single microfluidic design with or without sensors, capable of performing one unit operation.
Microfluidic platform	Single microfluidic design or combination of several systems with or without sensors, capable of performing more than one operation.
Prototype	System or platform, with or without sensors, at the end of Step III (see Figure 10) that may require further adjustments.
Microfluidic device or microdevice	Complete microfluidic system or platform (developed prototype).
Lab on a Chip	A device that integrates one or several laboratory functions on a single chip, while transporting and manipulating minute amounts of fluids (Wohlgemuth et al., 2015) at microliter scale.
Microstructured unit operation or system	Single unit operation that contains features with dimensions in the order of a micron.
‘Self-contained’ or standalone microfluidic system	Microfluidic system or platform that contains all the necessary components to facilitate a complete assay (from (Boyd-Moss et al., 2016)).
Modularization	“Designing with standardized units, dimensions or interfaces, which can be easily assembled, maintained as well as flexibly arranged and operated” (Weber, 2016).
Modular microfluidic system or microfluidic module	Microfluidic system that can be used as one part (module) of a microfluidic platform. Ideally, modular microfluidic systems present connectors that are compatible with many different other modules and also external equipment.
Modular microfluidic platform	Microfluidic platform that is composed of interchangeable microfluidic modules. The different modules may have different functions (act as different unit operations), but can be connected in any given order being compatible with other modules belonging to the same platform.
“Plug-and-play” microfluidic system	Modular microfluidic system with connectors enabling its easy connection with other modules (inspired by Lego® concept).
Multi-use or multi-purpose microfluidic system	Microfluidic system that may be used for different applications and/or different substrates and reaction conditions (e.g. with different samples, same sample but with a different purpose, with various flowrates, with various sensor strategies, etc.).
Multi-unit operation platform or device	Platform capable of performing more than one unit operation.
Process intensification	Process engineering approaches that result in a substantially smaller, cleaner, safer and more efficient technology (from (Buchholz, 2010)).
Scale-up	Increase of the overall dimensions of the unit operation or device by scaling its characteristic

	dimensions.
Parallelization or scale-out approach	Simultaneous operation of several chips of the same microfluidic system or platform in a parallel network.
Automation	Application of technology that allows the automatic operation and control, meaning with or without minimal human control, of a process.
Target analyte	Analyte (such as chemicals, biomolecules [e.g. DNA, proteins], whole cells, virus, particles [e.g. magnetic particles, dust, pollen]) present in the sample, that will be monitored in the system by the integrated sensor.
“Killer application”	“(…) product which has such highly desirable properties that it generates very large revenues with attractive margins in a comparatively short amount of time” (Becker, 2009).
Micro-plant or small-scale chemical production	Production process based on microfluidic or microstructured unit operations capable of producing compounds in the g per hour scale.
Industrial production plant or industrial production	Chemical or bio-based processes capable of producing and purifying a target compound in the multi-ton or several m ³ per hour scale.
Industry 4.0	Fourth industrial revolution focused on achieving smart industry and quality-by-design through integration of Internet of Things (IoT), in-house online data analytics and Big Data, more data and information exchange among stakeholders and plant-wide automation (Shrouf et al., 2014), (Hermann et al., 2016), (Stock and Seliger, 2016), (Weber, 2016), (Hofmann and Rüsch, 2017).
Enabling technology	Technology “that is used as a tool to solve a specific application problem” (Becker, 2009).

Highlights

- Overview of the current position of microfluidics in the chemical process and biotechnology market and discussion of some of the identified issues preventing its wider application.
- Presentation of a detailed guide to the development of microfluidic platforms, with its application in two hypothetical cases and three examples taken from literature.
- Short review on sensor technologies, materials and fabrication techniques used in microfluidics.
- Short review of essential modules for the development of modular microfluidics, and examples of developed microfluidic systems that can be used as such modules.
- Presentation of two models for commercialization of modular microfluidic platforms towards a wider acceptance and applicability.

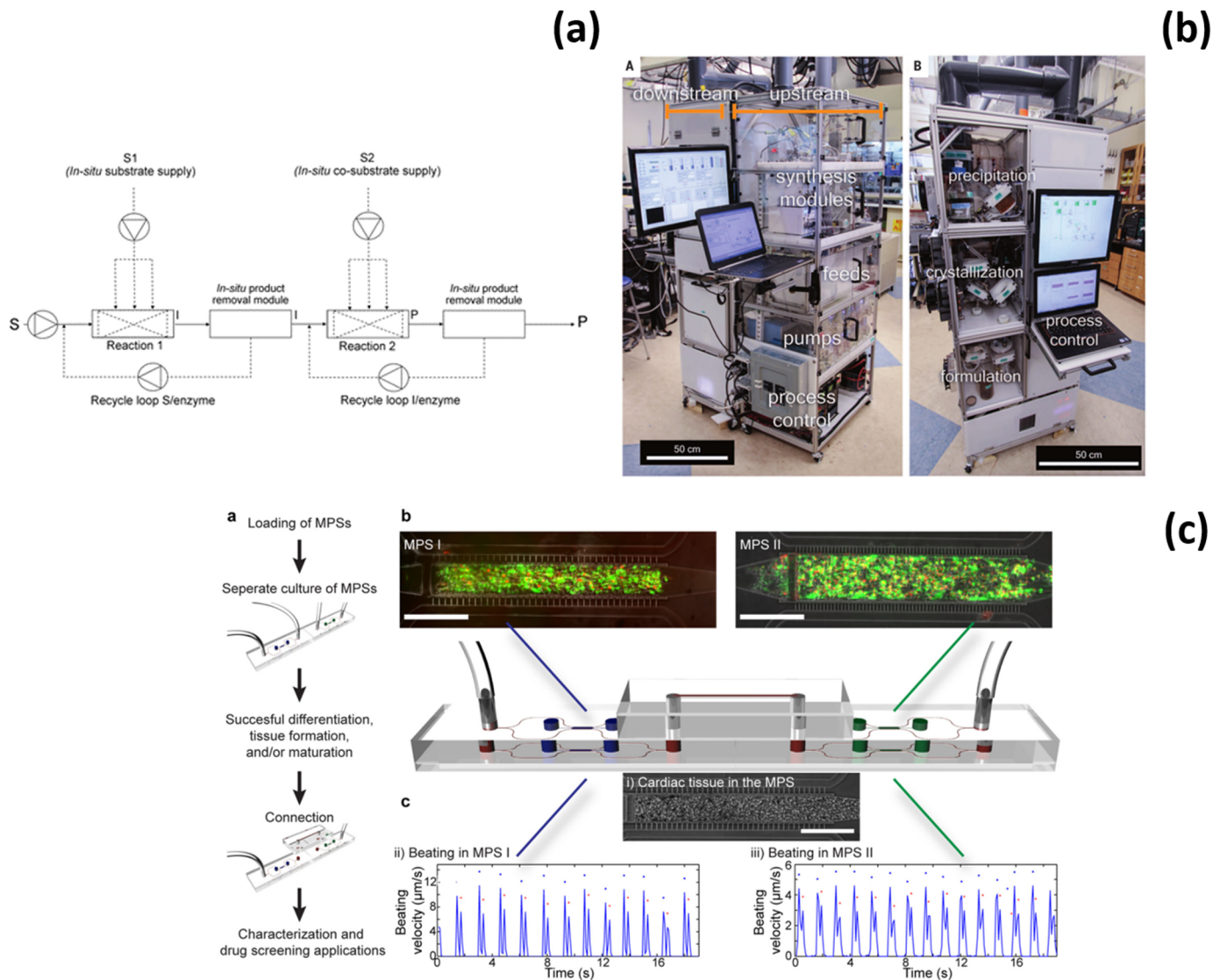


Figure 1

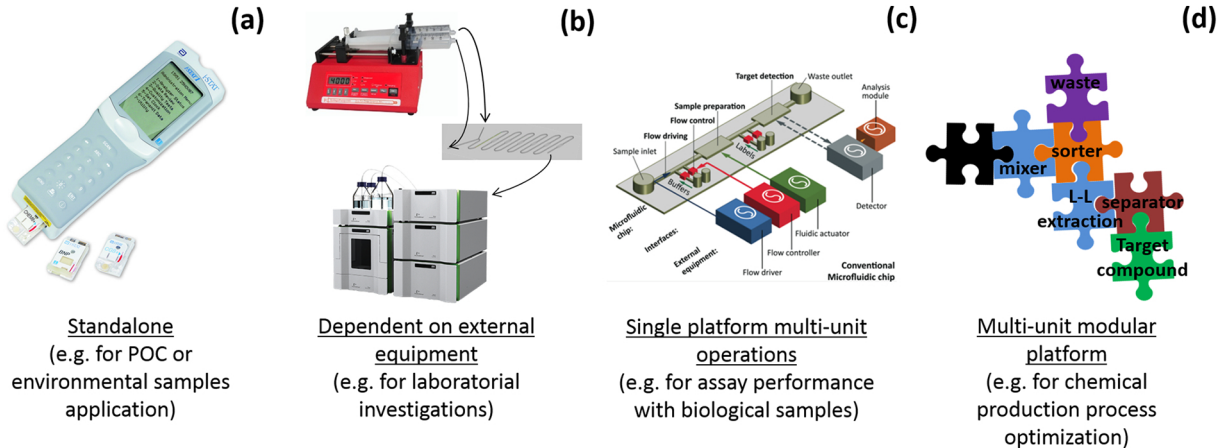


Figure 2

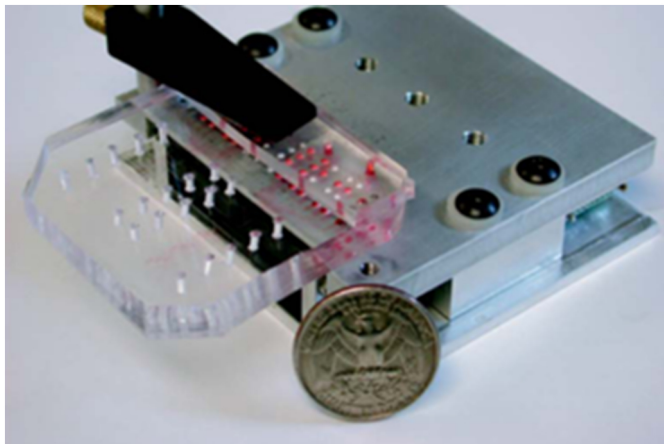


Figure 3

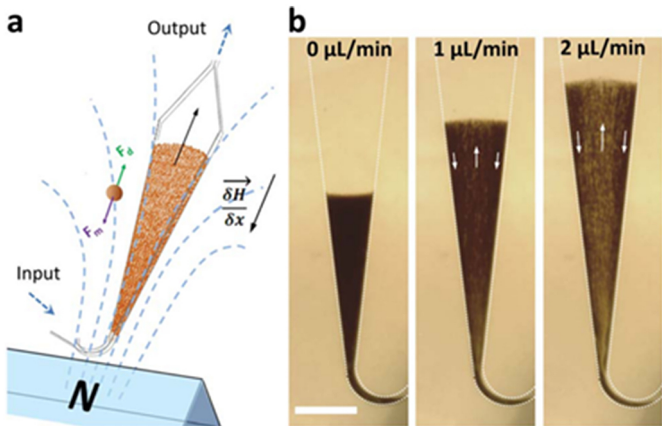


Figure 4

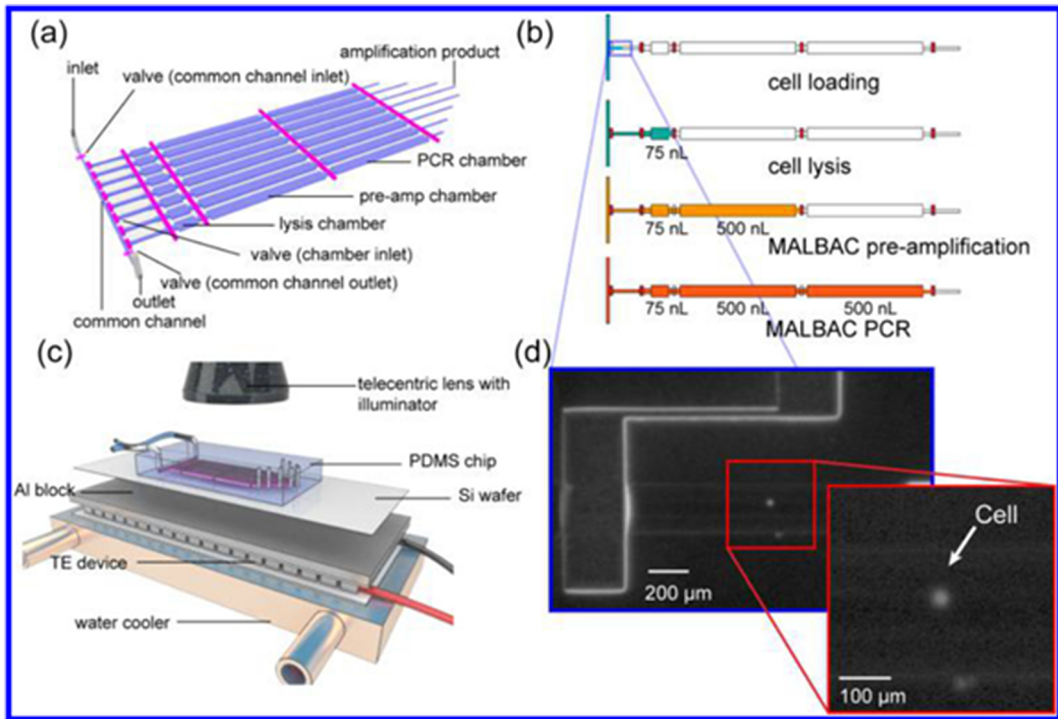


Figure 5

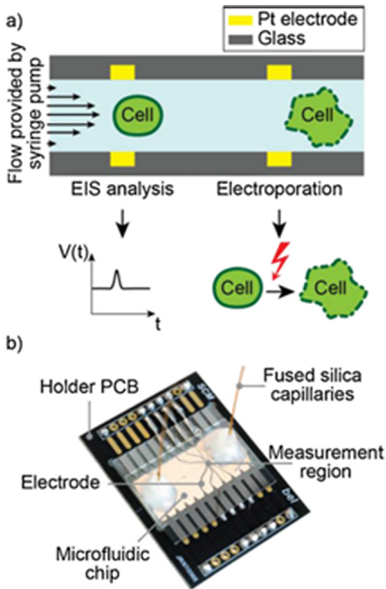
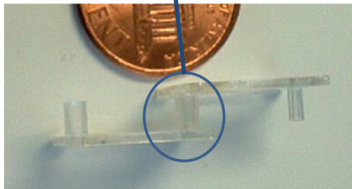
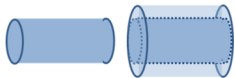


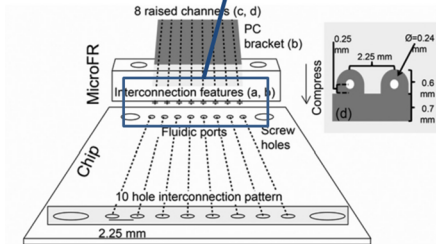
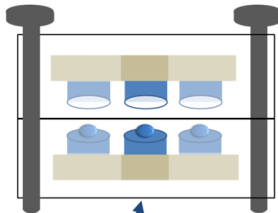
Figure 6

(a)



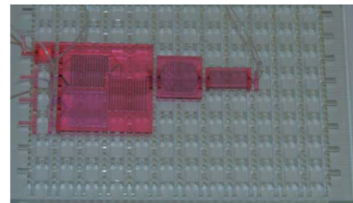
Pepper *et al.* (2007) [23]

(b)



Sabourin *et al.* (2013) [20]

(c)



Yuen *et al.* (2008) [24]

Figure 7

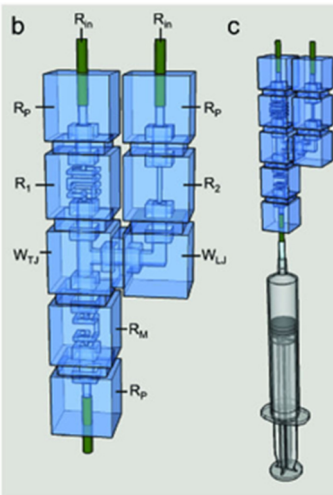
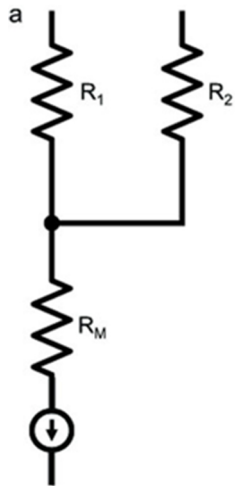


Figure 8



Origin of microfluidic modules	Fabricated by user based on design database (e.g. 3D printed)	and/or	Ready-to-use modules in a toolbox (standardized modules approach)
Module validation	<ul style="list-style-type: none"> With in-house available existing analytical devices; Original design developer provides guidelines for necessary tests within the design database; 		<ul style="list-style-type: none"> Provided pre-validated; Provided with operation parameters range; Provided with software and/or mathematical/fluidic model to guide assembly and use for each application;
Application	 <ol style="list-style-type: none"> 1. Fabrication (based on available technique or with method suggested in the database); 2. Assembly of the fabricated parts or to already existing systems; 3. Validation of assembled modular system; 4. Use in intended application. 		 <ol style="list-style-type: none"> 1. Assembly of the parts based on toolbox guide and intended application; 2. Run flow simulations considering intended application; 3. Use in intended application.
Users	Academics, Company R&I personnel, Prototype developers, Start-up personnel		Academics, Company R&I personnel, Prototype developers, Start-up personnel, Final device developers, General public
Technical expertise	High to medium		Low to medium technical expertise, especially if accompanied by modeling software
Field of application	Human and veterinary health diagnostics, Analytics, Biocatalysis, Chemical engineering, Organic synthesis, Biomedicine, Water and food quality, Resource recovery, Teaching (e.g. physical and transport phenomena, different unit operations), Sample (pre-) processing, Single cell phenomena, Sensor development.		

Figure 9

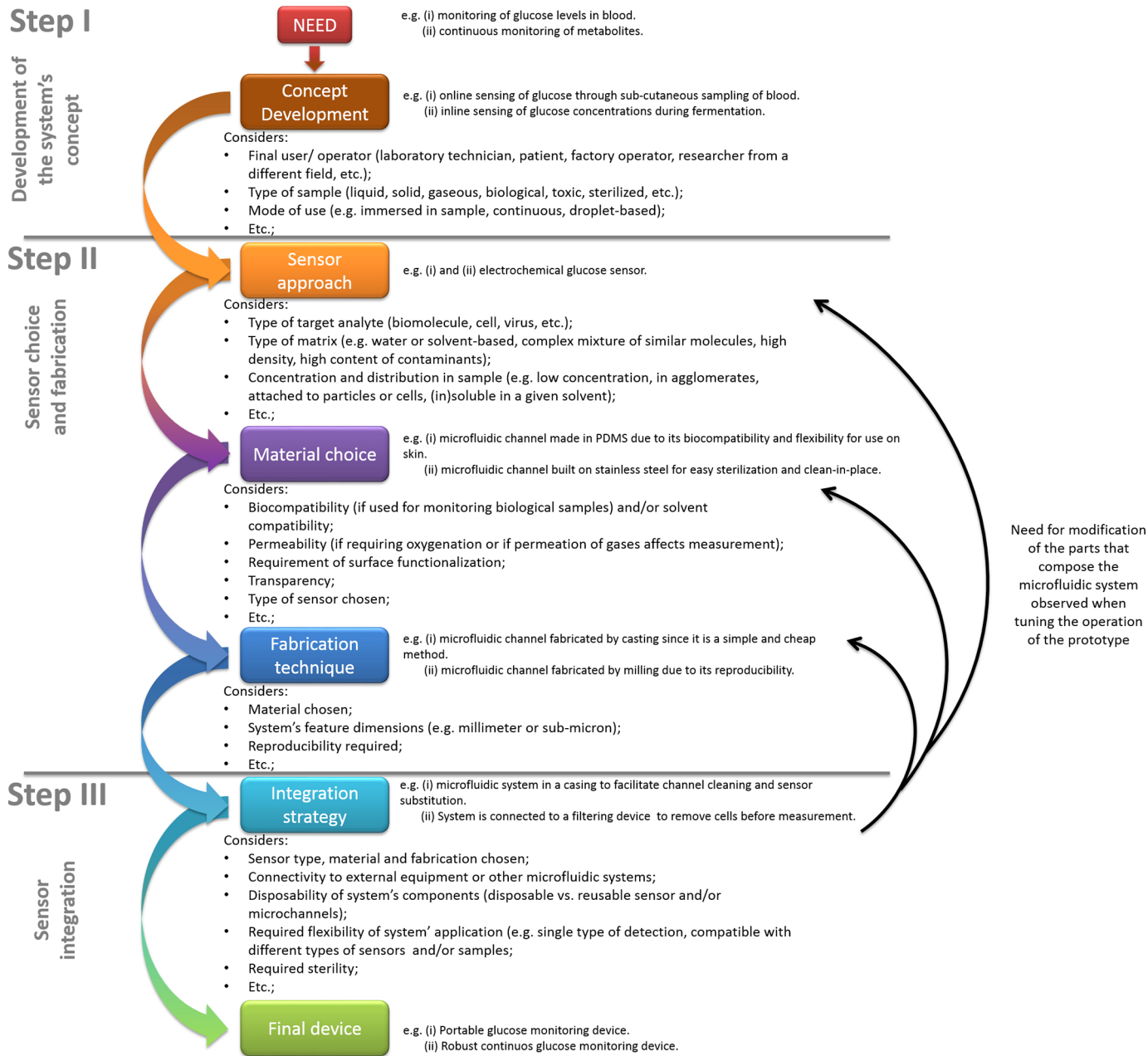


Figure 10

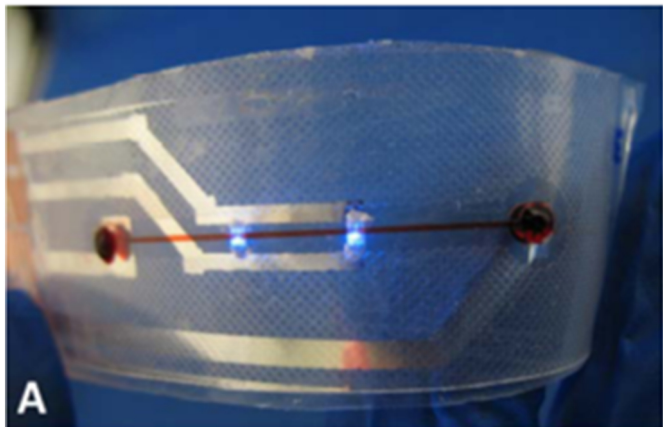
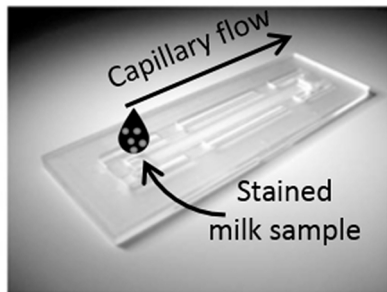


Figure 11

A.



B.



C.

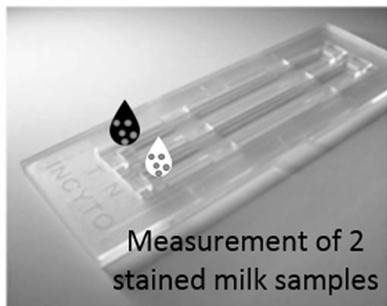


Figure 12

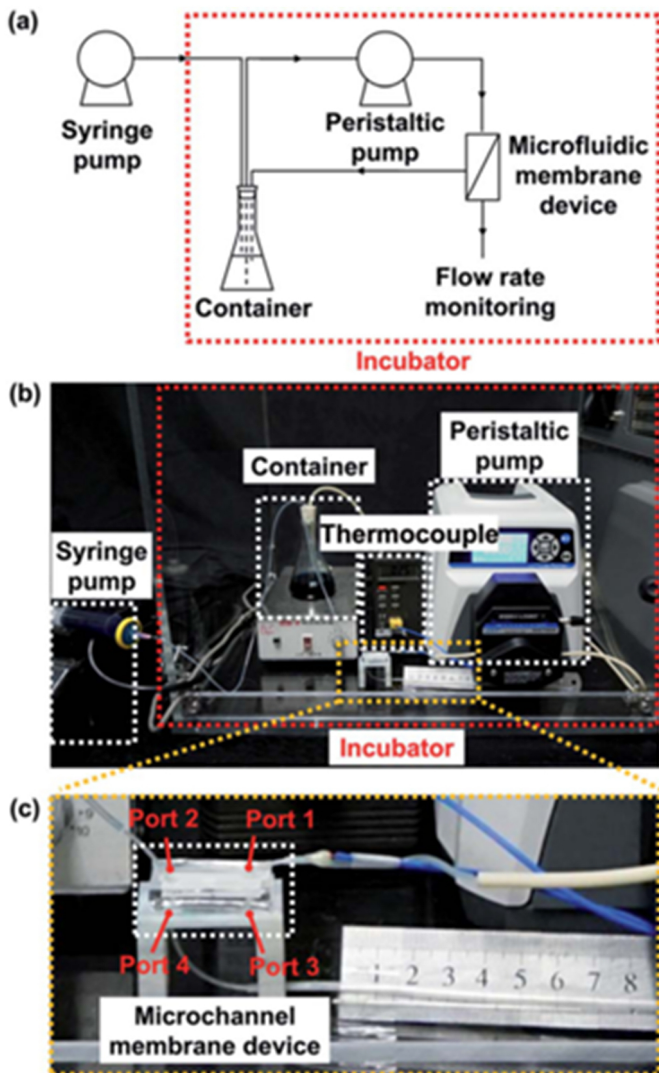


Figure 13